RESEARCH ANIMALS IN MEDICINE

RESEARCH ANIMALS IN MEDICINE

National Heart and
Lung Institute
National Institutes of Health

LOWELL T. HARMISON, Ph.D.
Editor

OCTOBER 2, 1973

DHEW PUBLICATION NO. (NIH) 72-333
U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE

Public Health Service
National Institutes of Health

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price $14.65
Stock Number 1740–0338

FOREWORD

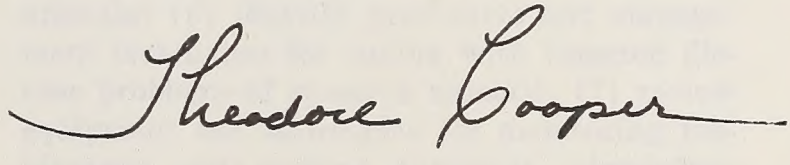
It is well known that the understanding and treatment of many human diseases has been enhanced by the study of laboratory animals in the past, and that the use of laboratory animals continues to be of major importance in medical research.

The current and growing use of animals in research has made it imperative that the benefits, conditions and limitations of such use be widely understood. To aid in increasing and disseminating information and understanding of this important aspect of research, the National Heart and Lung Institute (NHLI) sponsored a National Conference on Research Animals in Medicine.

I believe the Conference itself contributed a better understanding, among the 1000 participants, of many techniques and results of the use of animals and of some general features of animal models, and I hope that this book will be useful in bringing this information to the attention of an even wider audience.

I shall not attempt to summarize any of the technical findings or identify any that appear to be of major significance. One area that I do want to mention, however may seem to be "non-technical"—that dealing with animal care and welfare. I think it is very important to keep in mind the necessity of adequate care and humane treatment, not only because of legal requirements, but equally because of two other considerations: scientific validity and ethical concern.

The Conference and this text are one example of the continuing effort of NHLI to improve medical care and to disseminate information to the scientific community and to the public.



Theodore Cooper, M.D.
Director, National Heart and Lung Institute
National Institutes of Health

PREFACE

"Man, unlike any other thing, organic or inorganic in the universe, grows beyond his work, walks up the stairs of his concepts, emerges ahead of his accomplishments."

John Steinbeck, *The Grapes of Wrath*

In medicine, animal research is a critical path to accomplishment that permits us to grow beyond our current limitations and leads us to greater heights of knowledge and understanding of human health and disease.

Biological research is entering a new era in support of clinical medicine through greatly improved instrumentation and participation from the physical sciences. Along with this increased capacity for performance, there is an increased potential for well-defined and controlled animal experiments that focus on clinical problems. Critical emphasis must be placed on animal experiments, and these must involve the proper use and care of the laboratory animal. Advances in treatment and prevention of human disease are deeply rooted in animal research, which has provided a rich background of knowledge and insight. The developments of drugs and devices, as well as in techniques of application, are inseparably tied to *in vivo* studies. The results of these efforts are clearly reflected in those now living normal lives despite past devastating illness.

This book provides an additional step toward a better understanding of the role of animal research in medicine. It describes over 100 scientific investigations, the work of over 250 individual scientists in a variety of fields and includes the highly informative discussion engendered by the presentation of these investigations to the more than 1000 participants at the National Conference on Research Animals and Medicine, sponsored by the National Heart and Lung Institute in Washington, D.C. January 28–30, 1972.

The principal objectives are to: (1) review animal models simulating various cardiac, pulmonary, and systemic diseases; (2) identify pertinent physiological and biological data concerning the use of large animal species (e.g., dog, calf, sheep, minipig and primate) in medical research; (3) identify test-animal requirements for cardiopulmonary research and development, such as the testing and evaluation of circulatory or respiratory assist devices and techniques; (4) review current surgical techniques in animals (especially cardiovascular surgery and organ transplantation), including pre-operative care and preparation, techniques of anesthesia, and immediate and chronic post-operative care; (5) review some aspects of the pharmacology of anesthetics, cardiac drugs, and other agents in mammals (particularly ruminants and carnivores) as well as the genetic and biologic effects of irradiation on large animals; (6) identify preventive and management techniques for coping with common disease problems of research animals; (7) review equipment and techniques for monitoring respiratory and cardiac functions, circulatory dynamics, and other physiological alterations in animals, and to compare the data thus obtained with clinical data; and (8) provide a brief overview of guidelines and welfare requirements for care of research animals.

Scientific material addressed to these objectives is presented in 14 chapters. The first provides an overview of the role of animal research in clinical medicine; chapters II and III describe animal models of cardiac and pulmonary diseases, respectively, and chapter IV the use of animal models in surgery and transplantation. The next five chapters (V–X) describe contributions of fundamental disciplines (hematology, hemodynamics, physiology, pharmacology, anatomy and pathology, and nuclear medicine and genetics) to animal research. Animal diseases and animal resources are discussed in chapters XI and XII, respectively; monitoring techniques in chapter XIII. The final chapter is addressed to physical and ethical problems of animal care. Each chapter is more or less a

single entity with numerous ties to other chapters based on the discipline point of view being pursued. Several key aspects of the text are highlighted in the following paragraphs to permit scope and content to be brought into quick focus.

Animal models of acute myocardial infarction and cardiogenic shock have been valuable in the study of hemodynamics, myocardial metabolism and treatment of acute conditions by drugs or cardiac devices. However, these acute models do not provide opportunities to study the natural course of coronary atherosclerosis and its complications. The development of chronic models which will extend use of models to the study of the natural course of disease in various animal species and non-human primates is of prime importance. Various types of cardiac models using both closed and open chest techniques are discussed.

The development and evaluation of models of lung diseases, which is in an early phase, offers a significant opportunity for better definition and understanding of both normal and diseased lung performance. At this stage, particular emphasis has been placed on correlating clinical and physiologic aberrations with pulmonary morphology. Some of the pulmonary model studies have included attention to pulmonary hypertension, hypoxic-induced bovine pulmonary hypertension, pulmonary vascular disease (including medial hypoplasia of the pulmonary artery), acute respiratory insufficiency through induced fluid overload, alteration and study of the pulmonary micro-circulation and physiologic preparations utilizing perfused isolated lung. These studies as well as others may give insight into respiratory disease and function; and lead to better characterization and improved treatment and prevention techniques for man.

In the area of surgery and transplantation significant strides have been made in techniques involving anesthesia, ventilation, anti-arrhythmic regimes, implantation and post-operative techniques for many animal species. The information covered points up both the similarities and unique charcteristics of various animal species; e.g., dog, cat, pig, sheep, and non-human primates.

In animal research, the individual—and sometimes unique—features of each species must be taken into consideration. Four kinds of hemostatic mechanisms operate in animal species; blood coagulation, vaso-constriction, platelet agglutination, and the cross-linking reaction which occurs by means of the enzyme called plasmatransglutaminase. It should be noted that certain animals have only one of these mechanisms, and in case the mechanism fails, survival is not assured by any other compensatory mechanism. In many species, all four mechanisms operate and, although one may function abnormally, hemostasis may still be achieved. All four mechanisms function in man, hence, man has some degree of freedom regarding hemostasis. However, this increased freedom is associated with a greater probability of complications from thrombosis, a factor in finding suitable blood-compatible materials. It should also be noted that the study of blood groups in animals, despite their complexity, has yielded practical as well as theoretical results of immense value to the field. Further, animal colonies with both normal and abnormal blood systems provide significant opportunities to study and explore thrombosis and hemostasis.

The increased attention to prosthetic devices and biomedical instrumentation for monitoring has led to significant progress in defining and better understanding animal hemodynamics and extrapolating it to man. The information discussed deals with cardiovascular function in ponies, chimpanzees, horses, calves, and dogs. Although some of these species have not been studied as extensively as the dog, their specific physiological, pathological, genetic, and other characteristics make them appropriate for specific types of research models.

The role of laboratory animals in pharmacological studies with potential application to man has had a long history, and has raised many questions. In brief, when utilizing laboratory animals and extrapolating the data collected to man, one must have reasonable assurance that the drugs are handled and utilized in the species under study as they would be in man; otherwise, serious questions may arise concerning the applicability of the animal studies. For example, where there are marked differences in the techniques of handling the drugs in one or in several

species, the efficacy of the drugs come under question. Any differences in technique or handling procedures must be compensated for by the development of relevant toxicity data, if these drugs are considered for use in man. The discussion identifies limitations and advantages in utilizing numerous animal species in pharmacological investigations and the difficulty in extrapolating animal results to man. Particular attention is directed to the differences in metabolic rates among animal species. Recognition of these variations will strengthen the data base in animal investigation when testing new pharmacological agents intended for use in man.

The classic disciplines of anatomy and pathology continue to provide valuable information, new approaches, and fresh insights into a number of diseases and important problems associated with various disease. Genetic and biological effects of radiation on large animals, and how such models have served to provide data relevant to man, are reviewed. In assessing these effects of radiation, it is of overriding importance that the quality of radiation, its dose rate, and dose to the susceptible tissue be carefully defined. Moreover, these data must be viewed in the light of other available information about the effects of radiation in man and animal.

One cannot overemphasize the importance of animal diseases in animals used in medical research. Their effects can mask or modify the course of other diseases being studied. Those concerned with laboratory animal medicine fully realize the import of specific animal diseases and their possible pertubating effect upon the principal studies being conducted in the animal. Research studies involving unhealthy animals not only have limited or no value, but can waste both time and resources. The discussions include consideration of animal diseases that are useful in medical research as well as those which are detrimental when the animals are used in research and in the formulation of specific types of animal models.

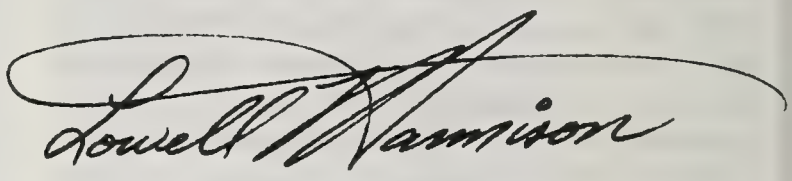
In discussions of animal resources, the principal attention is directed to coping with such basic problems in the use of animals as their highly variable and unknown genetic background, age, temperament, and disease status. To some extent, it was felt that these problems, as well as others, could be overcome through the use of animals from more standardized colonies in which baseline data pertaining to anatomy, physiology, hematology, biochemistry, etc. for specific sex, age, and other groups would be available. Some investigators have been reluctant to use the non-human primate because of unavailability of properly conditioned animals, inadequate knowledge of the primate, of methods for their maintenance and handling, of appropriate equipment and of trained animal technicians. Obviously, these problems have made it difficult for small colonies to be maintained by single investigators for their own study purposes. However, some of these problems are now being overcome through the primate research centers and institutional primate resources that contribute to research economy and conservation of animals. Further, it should be noted that there exists a primate information center which can provide normative data and bibliographic services on primates and their use in research.

Modern instrumentation and techniques of application are making possible great strides in the acquisition of information on anesthetized and unanesthetized animals under acute and chronic conditions. The topics discussed range from engineering details of instrumentation through new knowledge of cardiovascular, respiratory and other diseases derived by use of new instrumentation. An important element to note is that greater emphasis is being applied to the importance of making measurements of physiological variables in the unanesthetized animal with all normal control mechanisms intact. This is in contrast with the more traditional work on the anesthetized animal or excised tissue preparations in the past. It is apparent that the development and application of atraumatic instrumentation will have a significant, conceivably revolutionary, impact on disease diagnosis, monitoring and treatment.

Of vital importance to the field of animal research are effective guidelines and legislation to assure the proper treatment and welfare of research animals. And, equally important, is the interpretation, communication, and understanding of the various facets of these require-

ments. The discussion is directed to the regulations required by law, the recommendations included in the NIH Guidelines, implementation of these guidelines, and the practical considerations in achieving improved laboratory investigations. It is apparent from the discussions and information presented by both the research investigator and those interested in animal welfare that enormous strides have been made in bringing into balance the various approaches and philosophies concerning animal research.

I hope that the conference on research animals in medicine and this text will stimulate new, imaginative, and creative efforts in this field, and foster contacts and interactions between those who search for knowledge and those who must use the results in the treatment and prevention of disease.



Lowell T. Harmison, Ph.D., Editor
Special Assistant to The Director
National Heart and Lung Institute
National Institutes of Health

SCIENTIFIC MANUSCRIPT REVIEW BOARD AND CONFERENCE SESSION CHAIRMAN

Lawrence S. Cohen
Yale University School of Medicine
New Haven, Connecticut

Eugene P. Cronkite
Brookhaven National Laboratory
Upton, New York

Dean Franklin
Scripps Clinic and
Research Foundation
La Jolla, California

Robert L. Hamlin
Ohio State University
Columbus, Ohio

Lowell T. Harmison
National Heart and Lung Institute
Bethesda, Maryland

Edward W. Hawthorne
Howard University
Washington, D.C.

D. D. Joel
Brookhaven National Laboratory
Upton, New York

Michael P. Kaye
IIT Research Institute
Chicago, Illinois

Paul Kezdi
Cox Heart Institute
Kettering, Ohio

Bert K. Kusserow
University of Vermont
Burlington, Vermont

C. Grant LaFarge
Children's Hospital Medical Center
Boston, Massachusetts

Claude Lenfant
National Heart and Lung Institute
Bethesda, Maryland

Charles McPherson
National Institutes of Health
Bethesda, Maryland

Thomas C. Militano
National Heart and Lung Institute
Bethesda, Maryland

John C. Norman
Harvard Medical School
Boston, Massachusetts

Joseph E. Pierce
National Heart and Lung Institute
Bethesda, Maryland

Walter H. Seegers
Wayne State University
Detroit, Michigan

Frank J. Veith
Montefiore Hospital
New York, New York

Homer Warner
University of Utah
Salt Lake City, Utah

ACKNOWLEDGEMENTS

The editor would like to express his deep appreciation to the authors for their excellent scientific manuscripts and for their generous contribution of time and energy in manuscript preparation, delivery and review for publication. A special word of thanks is extended to the scientists who served on the manuscript review board and as session chairmen for their outstanding contribution to the conference and publication.

The editor is grateful to many members of the National Heart and Lung Institute's staff, in particular the staff of the Medical Devices Program, for their unfailing support and assistance. They worked diligently in their planning and organizing activities for the conference, and carried a significant burden in making arrangements for the conference.

The editor wants to acknowledge and express sincere appreciation to the Peat, Marwick, Mitchell Company and their supporting personnel for their excellent support and cooperation in assisting with the papers and discussions of the conference.

Further, the editor wants to thank all those who read and commented on the many scientific manuscripts at the various stages of publication.

TABLE OF CONTENTS

	Page
FOREWORD	iii
PREFACE	v
REVIEW BOARD	ix
ACKNOWLEDGEMENT	x
TABLE OF CONTENT	xi
CHAPTER I—INTRODUCTION	
The Role of Animal Research in Clinical Medicine	
Robert Van Citters	3
CHAPTER II—CARDIAC MODELS	
Animal Models of Atherosclerosis	
Thomas B. Clarkson	11
Animal Research in Shock	
R. M. Hardaway, III	21
Observations on Experimental Myocardial Infarctions in Dogs	
Jacob I. Hirsch, Fritz Streuli and Fred Gorstein	29
Animal Models of Myocardial Infarction: Current Limitations and Future Promise	
W. B. Hood, Jr. and J. C. Norman	41
Turkey Round Heart Disease: A Model Cardiomyopathy	
E. F. Jankus, N. A. Staley and G. R. Noren	53
Studies of a Cardiogenic Shock Model	
P. Kezdi, S. N. Misra, R. K. Kordenat, and T. J. Smith	61
Biventricular Mechanical Bypass; Physiological Studies During Induced Ventricular Failure	
C. Grant LaFarge and W. F. Bernhard	75
Model of Advanced Coronary Atherosclerosis, Myocardial Infarction and Sudden Death with Lethal Arrhythmias in Swine	
K. T. Lee, J. Jarmolych, D. N. Kim and W. M. Lee	93
Experimental Myocardial Infarction in Nonhuman Primates	
M. R. Malinow, J. D. Hill, M. Lees, W. P. McNulty and A. J. Ochsner, III	105
Canine Coronary Insufficiency Models for Evaluating Systems for Circulatory Support	
J. Rosensweig and S. Chatterjee	119
Pulmonary Artery Constriction in the Cat: A Model for Ventricular Hypertrophy and Congestive Heart Failure	
J. F. Spann, Jr. and G. M. Lemole	141
Experimental Myocardial Infarction by Closed Chest Technique	
E. L. Stanley, P. Kezdi and R. K. Kordenat	157
Left Ventricular Failure and Cardiogenic Shock Following Acute Myocardial Infarction: A Correlation of Clinical and Experimental Observations	
K. T. Weber, R. A. Ratshin, C. E. Rackley and R. O. Russell, Jr.	169
Proteases and Chemical Mediators in Acute Coronary Occlusion in the Dog	
H. J. Wilkens, R. Steger and N. Back	179

Page

CHAPTER III—PULMONARY MODELS

Pulmonary Hypertension in Young Macaca Arctoides Monkeys: A Sequela to Monocrotaline Intoxication
J. R. Allen, J. M. Jenny and C. F. Chesney 187

Hypoxic-Induced Bovine Pulmonary Hypertension
A. F. Alexander, C. S. Card, R. S. Jaenke, J. L. Hicks and D. H. Will 193

A Model for Closed Thoracic Observation of the Pulmonary Microcirculation in Shock
W. A. Cook, P. B. Speiller, H. Sherman and S. K. Klausner 201

A Pulmonary Xenograft Model for Microcirculatory Observation
W. A. Cook, P. B. Speiller, S. K. Slausner, S. B. P. Sinha, Y. Kikkawa and F. J. Veith 209

Acute Bovine Pulmonary Emphysema: Evaluation of Tryptophan Induction
R. A. Kainer, C. S. Card and G. P. Epling 217

Chronic Obstructive Lung Disease in Horses
J. R. Gillespie and W. S. Tyler 223

Pathophysiologic Assessment of a Canine Model of Induced Acute Respiratory Insufficiency
Robert H. Liss and John C. Norman 229

Medial Hyperplasia of the Pulmonary Arteries of Cats
T. W. Swerczek 237

Alveolar, Bronchiolar and Pulmonary Vascular Responses of Isolated Rat and Guinea Pig Lungs
Frederick Sperling, William L. Marcus and A-A. O. Coker 243

The Effect of Hypoxia on the Pulmonary Microcirculation
W. W. Wagner, L. P. Latham and D. R. Halsey 253

Cinemicroscopy of the Pulmonary Microcirculation in Shock
Watts R. Webb, Stennis D. Wax, K. Kusajima, Frederick B. Parker, T. M. Kamiyama and T. Murakami 261

Models of Cardiopulmonary Function in Calves
J. A. Will, G. E. Bisgard, A. V. Ruiz and R. F. Grover 267

CHAPTER IV—SURGERY AND TRANSPLANTATION

Implantation Surgery of Artificial Hearts
T. Akutsu, H. Takagi and H. Takano 277

Experimental Cardiac Surgery in the Calf
William J. Donawick and James W. Buchanan 289

Regional Denervation of the Canine Heart
W. P. Geis and M. P. Kaye 295

Surgical Experience with Nonhuman Primate Animals 1963–1971
E. I. Goldsmith 313

Lung Transplantation in Dogs
James D. Hardy 323

Experimental Aorto-Coronary Direct Revascularization in the Dog
Gerard S. Kakos and H. Newland Oldham, Jr. 347

Total Cardiovascular Bypass with Membrane Oxygenator in Calves
M. P. Kaye and J. B. Pace 355

Preservation of Organs for Transplantation
Theodore I. Malinin 365

Pancreas Transplantation in Dog and Man
F. K. Merkel 375

Page

Left Ventricular Assist Devices in the Calf: Anesthesia, Ventilation, Antiarrhythmic Regimen, and Implantation
Farouk A. Molokhia, Fred N. Huffman, William J. Robinson, Panayiotis J. Asimacopoulos, Ronald B. Ponn and John C. Norman 383

The Baboon in Medical Research with Particular Reference to Kidney Transplantation
G. P. Murphy 393

Preconditioning, Implantation and Postoperative Care of the Artificial Heart Research Calf
J. L. Peters, F. M. Donovan, J. Kawai, C. S. Kwan-Gett, H. H. J. Zwart and W. J. Kolff 399

The Use of Large Animals in Experimental Cardiovascular Surgery
J. N. Ross, Jr., J. M. Fuqua, Jr., B. S. Ruark and J. H. Kennedy 417

Left Ventricular Hemodynamic and Dimensional Responses to Treadmill Exercises in Normal and Cardiac Denervated Dogs
E. B. Stinson, G. Rahmoeller, P. L. Tecklenberg, S. B. Colvin, K. W. Jones and J. E. Pierce 429

Single Lung Transplantation with Immediate Ligation of the Opposite Pulmonary Artery in the Dog: A Model for Assessing the Functional Adequacy of Transplanted Lungs
F. J. Veith, S. B. P. Sinha, S. S. Siegelman and J. W. C. Hagstrom 437

CHAPTER V—HEMATOLOGY

Blood Grouping Systems in Dogs, Cattle and Sheep
E. B. Black and J. M. Stengle 449

Factors Regulating the Production of Coagulation Activities in Perfused Organs
W. J. Dodds and L. W. Hoyer 463

Hemostasis in the Animal Kingdom
K. Laki 473

Blood Groups of Apes and Monkeys: Human and Simian Types
J. Moor-Jankowski 483

Sheep Blood Coagulation Values
M. L. Povar 489

Experimental Approaches to the Study of Vitamin K
Walter H. Seegers 493

Summary of Contribution of Hemophilic Dogs to Knowledge and Human Welfare
Kenneth M. Brinkhous 501

A Survey of Blood Groups in Several Species of Large Animals Used in Medical Research
Clyde J. Stormont 505

CHAPTER VI—HEMODYNAMICS

Cardiovascular Hemodynamics in Domestic Ponies
James F. Amend, Harold E. Garner, John P. Rosborough and Hebbel E. Hoff 517

Comparative Mechanical Blood Properties
P. L. Blackshear and R. J. Forstrom 525

The Effects of the Catecholamines and Glucagon on the Performance of the Equine Heart
W. C. Bowie, R. C. Purohit, D. E. Adams, K. T. Somaiah, E. W. Hawthorne and J. E. Hinds 541

Page

An Analysis of Left Ventricular Dimensional Changes in Conscious Animals
L. N. Cothran, E. W. Hawthorne and H. Sandler 553
A Method to Study the Regulation of Coronary Flow and of Other Cardiovascular Functions in the Unanesthetized Performing Dog
Walter Ehrlich, Julij G. Tosheff, Paolo Caldini, David Krausman, Francine Schrijen and Joseph V. Brady 567
Rheologic Properties of Blood Vessels
Joseph S. Janicki, Dali J. Patel, John T. Young and Ramesh N. Vaishnav 573
Requirements for Long-Term Cardiopulmonary Bypass Circulation: Hypotheses and Data From Research Animals
R. B. Shepard, E. I. McGowan, J. W. Scott, H. J. Baker, D. M. Bowen and K. M. Pruitt 583
Hemodynamics in the Chimpanzee: Unanesthetized and Anesthetized
H. L. Stone and H. Sandler 593

CHAPTER VII—PHYSIOLOGY

Comparative Electrocardiography
Robert L. Hamlin and C. Roger Smith 609
Physiologic and Pharmacologic Response of Chronically Denervated Feline Hearts
Kenneth M. Kent, Peter J. Dempsey, Zena T. McCallum and Theodore Cooper 619
"Pacing" Left Ventricular Function Curves in Conscious Dogs
F. Kraft-Hunter and E. W. Hawthorne 625
Electrocardiogram, Myocardial Action Potential and Cardiac Excitability in Laboratory Animals
E. Lepeschkin 647
An Animal Model of Isorhythmic Atrioventricular Dissociation
M. N. Levy 655
Pulmonary Function Testing of Unanesthetized Beagle Dogs
J. L. Mauderly and J. A. Pickrell 665
Changes in Left Ventricular Hemodynamics and Dimensions During Rejection of the Orthotopically Transplanted Canine Heart
E. B. Stinson, P. L. Tecklenberg, J. F. Hollingsworth and K. W. Jones 681

CHAPTER VIII—PHARMACOLOGY

The Pharmacokinetics of Amphetamine in Domestic Animals
J. D. Baggot and L. E. Davis 691
The Use of the Animal Model in Assessing Analgesic Potency and Dependence Liability
J. Cochin 701
Pharmacologic Actions of Newer Beta Blocking Agents
Lawrence S. Cohen, George F. Vastagh and Jere H. Mitchell 709
Comparative Pharmacokinetics in Domesticated Animals
L. E. Davis, C. A. Davis and J. D. Baggot 715
The Actions of Cardioactive Drugs on Developing Myocardium
William F. Friedman 735
Drug Studies on Isolated Animal and Human Cardiac and Vascular Tissues: Advantages and Limitations
Joseph V. Levy 749
A Study of Cell Kinetics in the Rat Thymus After Perturbation by Dexamethasone
J. C. Pierce 755

Page

Application of Miniature Swine to Anesthetic Studies on the Cardiovascular System and Hepatic Inhalation Anesthetic Metabolism
D. C. Sawyer, E. I. Eger, II and W. V. Lumb 759

CHAPTER IX—ANATOMY AND PATHOLOGY

A Correlative Study of the Anatomy of the Cardiovascular Systems of Animals and Man
Wesley D. Anderson 767

Direct Visualization of the Coronary Microcirculation of the Left Atrium of the Cat
R. J. Bing and K. D. Hellberg 807

Cardiac Effects of Hemorrhagic Shock in Dogs and Other Animals
D. B. Hackel, J. Chang, N. B. Ratliff and E. Mikat 813

Pathological Techniques Applied to Artificial Organ Research. An Animal Model for the Detection of Surface-Induced Thrombosis and Embolism
B. K. Kusserow 817

Spontaneous Cardiopulmonary Disease in Nonhuman Primates: Potential Models
W. P. McNulty 829

Arteriosclerosis and Other Vascular Diseases in Zoo and Laboratory Animals
C. Stout and F. Bohorquez 841

Alterations in DNA Synthesis and Other Metabolic Processes in Aortas of Cholesterol-Fed Swine
W. A. Thomas, R. A. Florentin, S. C. Nam, J. M. Reiner and K. T. Lee 861

CHAPTER X—GENETIC AND BIOLOGICAL EFFECTS OF RADIATION

Radiation Studies with Swine
D. G. Brown 875

Extracorporeal Irradiation of Blood and Lymph
D. D. Joel and E. P. Cronkite 887

Preliminary Data on Chromosome Aberrations in Swine and Human Leukocytes with a Brief Review of Factors Affecting their Observed Frequency
F. P. Hungate and B. J. McClanahan 899

Biological Effects of Radiation from Simulated ^{238}Pu Power Sources in Dogs
F. A. Kallfelz, C. L. Comar, P. H. Craig and A. P. Casarett 905

Observations on Chromosomal Morphology During Simulated Intracorporeal Radiation from Nuclear-Powered Artificial Hearts
Farouk A. Molokhia, William J. Robinson, Fred N. Huffman and John C. Norman 911

Pathologic Effects of Chronic ^{90}Sr Ingestion in Miniature Swine
H. A. Ragan, P. L. Hackett, B. J. McClanahan and W. J. Clarke 919

A Four-Year Study of Long-Term Effects of Implanted Discrete Heat and Radiation Sources in Dogs and Primates: Preliminary Studies Leading to Implantable Nuclear-Fueled Circulatory Support Systems 931
G. W. Sandberg, Jr., F. A. Molokhia, P. R. Ponn, F. N. Huffman and J. C. Norman

Animal Radiation Data and their Relevance to Man
Harry E. Walburg, Jr. 939

CHAPTER XI—ANIMAL DISEASES

Experimental Viral Valvulitis
N. P. DePasquale and George E. Burch 945

Respiratory Diseases of Dogs, Calves, and Sheep
R. A. Griesemer and R. L. Farrell 951

Page

Virus Infections of "Normal" Healthy Animals
G. D. Hsiung and N. S. Swack 961
Significance of Simian Viruses
S. S. Kalter 965
Dirofilaria Immitis: A Review of Present Knowledge
G. Pacheco 977
Diseases Encountered During the Conditioning of Random Source Dogs and Cats
A. E. Palmer 981
Congenital Defects of the Cardiovascular System in Dogs
D. F. Patterson 991
Spontaneous Diseases in Germfree Animals
Morris Pollard 1005
Resistance of Swine to Bordetella Rhinitis
W. P. Switzer 1015

CHAPTER XII—ANIMAL RESOURCES

Establishment, Organization, Management and Functions of an Investigative and Diagnostic Laboratory within the Department of Experimental Animal Medicine at the University of Washington
W. C. Dolowy and L. J. Swango 1021
Special Considerations in the Use of Calves for Cardiovascular Implant Studies
F. T. Galysh and R. F. Wallin 1033
Improved Resource Utilization Through Animal and Facilities Sharing at the Laboratory for Experimental Medicine and Surgery in Primates
E. I. Goldsmith, J. Moor-Jankowski and J. Davis 1047
The Role of Veterinary Diagnostic Support Laboratories in a Research Animal Colony
A. M. Jonas 1055
The Research Dog: Random Source or Colony Reared?
S. Potkay and J. D. Bacher 1061
A Support Laboratory for a Medical School Animal Research Facility
Ward R. Richter 1067
Progress in the Development of a Histocompatible Dog
J. W. Templeton, A. L. Rogers and W. S. Fletcher 1073
The Primate Information Center as a Research Tool in Studies of the Heart and Lung
Maryeva W. Terry 1081

CHAPTER XIII—MONITORING

Gamma-Ray Densitometry in the Analysis of Hemodynamic Function
J. D. Cohn, K. Ito and L. R. M. Del Guercio 1085
The Use of a General-Purpose Time-Shared Computer in Physiology Research
E. Dong, G. C. M. Wiederhold 1091
Biotelemetry Transmitter Design Methods
V. G. Ellerbruch, F. C. Fitchen and R. W. Sawrey 1105
Measurement and Radiotelemetry of Cardiovascular Variables in Conscious Animals: Techniques and Applications
Dean Franklin, Stephen F. Vatner, Charles B. Higgins, Thomas Patrick, W. Scott Kemper and Robert L. Van Citters 1119
Implant Telemetry and Stimulation
Wen H. Ko 1135

Page

Capabilities of Implantable Transducers
Eph Konigsberg 1145
A Mathematical Model for Measuring Blood Flow by Residue Detection When Radiotracer Recirculation Interferes
K. B. Larson and D. L. Snyder 1151
Pulsed Doppler Ultrasonic Flowmetry
D. Y. Loisance, J. P. Lenriot and P. A. Peronneau 1161
Implantable Ultrasonic Blood Flowmeters
James D. Meindl 1167
Percutaneous Conduction of Electrical Signals: Interface Techniques and Tissue Reaction at Electrode Site
J. T. Mortimer and P. H. Peckham 1187
The Use of Implantable Telemetry Systems for Animal Monitoring
H. Sandler, T. B. Fryer and H. L. Stone 1197
The Adaptive Control of Therapeutic Procedures
N. P. Thompson, B. Widrow and C. Schade 1207
Real-Time, Time-Sharing Computers in the Animal Laboratory
W. S. Topham 1217
Review of Animal Instrumentation: Transducer Capability
W. S. Topham 1229
A Multi-Station Computer System for Acquiring and Processing Animal Data
Eugene F. Uretz, John A. McClary and Steve Morgan 1243
The Conscious Calf Preparation: Technical Aspects of Surgery, Animal Care, Implanted Instrumentation and Monitoring for Cardiovascular Data Acquisition
K. T. Weber, J. S. Janicki, A. A. Walker and B. H. Dennison 1259

CHAPTER XIV—THE USE OF ANIMALS IN MEDICAL RESEARCH AND EXPERIMENTATION
Lowell T. Harmison 1271
Animal Welfare Act (Public Law 91–579) and Amendments
D. F. Schwindaman 1271
NIH Animal Care Guidelines
Mark Conner 1274
Panel Presentations
Charles McPherson 1277
Joseph E. Pierce 1277
John C. Norman 1280
Julius Cass 1282
Harold Parker 1283
Open Discussion 1285

CHAPTER I

INTRODUCTION

The Role of Animal Research in Clinical Medicine

THE ROLE OF ANIMAL RESEARCH IN CLINICAL MEDICINE*

Robert Van Citters**

INTRODUCTION

The objectives of the papers presented in this book touch on many aspects of research and medicine. It is significant that they are target-oriented. They speak to the attainment of a practical rather than a philosophical goal. The topic which I shall discuss, "The Role of Animal Research in Clinical Medicine," is also very broad and it too speaks to the practical application of research results.

With topic of this latitude, one is immediately tempted to focus remarks upon the justification of animal research through a detailed review of the past contributions to clinical medicine and to humanity. The fact that almost a thousand biomedical scientists attended the meeting negates any need to justify its theme. All of us have been influenced both in our individual lives and in the conduct of our research careers by the direct application of results of animal research in the past. Many, if not most, of the great chapters in the history of medicine have evolved from direct application of results of animal research to clinical medicine. They bring to mind the great names in the history of medicine—Galen, Harvey, Pasteur, Leeuwenhoek, Koch, and Jenner—just to mention a few. It is worth noting in passing that the applications of research have been extended well beyond clinical medicine. Man's conquest of the air and ultimately of space was preceded by animal experiments. Three common farm animals made the first balloon ascension long before man ventured into the air. A broad variety of animal subjects pioneered in the development of high altitude technology before man went into the stratosphere and, more recently, a whole menagerie of beasts sampled the outer atmosphere in advance of man's first orbital flight. I should also point out that each advancement in medical research also manifests itself in improvements in clinical veterinary practice. The results of laboratory studies have inevitably benefited animals as well as humans because veterinary practice tends to mimic clinical practice. In veterinary practice, antibiotics and hormones, dietary supplements, surgical procedures, and complicated diagnostic procedures that are common today are all borrowed from clinical medicine.

The objectives of animal research then are varied. Some are purely descriptive or phenomenological; some are accomplished purely for the sake of research; some are applied research in the technological or veterinary sense; but for the most part, researchers have studied the animal in order to extrapolate to man. They have recognized in the experimental animals an analog of man and have modified the animal to become a model of man or a model of diseased man. The ultimate goal has been the application of results obtained from animals in order to improve the welfare of man. The question then is how to improve or extend this role or how to facilitate the application of information derived from animal research to the solution of problems of human disease. Since this question is a very broad one, I will only suggest a few answers here.

I would like to address myself first to the general issue of animal models. The concept of animal models has become so commonplace in our thinking that it occupies an almost reflex role in the formulation of our experimental designs. Actually, herein lies a problem. Rigorous definition of the word "model" varies some-

* This was the keynote address at the National Conference on Research Animals in Medicine.

** Dean, School of Medicine, University of Washington, Seattle, Washington.

what from the context in which most of us have generally used the term. A model, as the dictionary would define it, is a small copy, an imitation or a preliminary representation which serves as the plan from which a final larger object is constructed or formulated. A model then is intended to be copied or followed. I point this out because it is at variance with the common practice in animal research. Most animal models are not, in fact, an accurate representation of the larger object, which in most cases is man. I can put it another way and more bluntly, that a staggering amount of medical research is accomplished on inappropriate models. This has been responsible for an enormous duplication of effort, confusion of results and often plain misunderstandings.

For example, in cardiovascular research, consider that the animal which has attracted the most effort and has been studied in the greatest depth in research related to the cardiovascular systems is the mongrel dog. It would be inappropriate for me to demean the contribution which studies on the dog have made to our overall fund of knowledge, but I think it is entirely appropriate to point out that there has been an amazingly small effort to determine the validity of the dog's cardiovascular system as a model for that of man. The dog came to occupy its present prominent position due to largely nonscientific reasons. The mongrel dog is available, cheap, convenient, and tractable, and I suppose that it continues to be used for all of these reasons. But tractability, cheapness, availability and convenience are really not bona fide criteria for selection of a model. Other, more appropriate models do exist, but many investigators have been unwilling to look beyond the dog. I see a need to break away from the tendency to use as models only those species which have historically enjoyed popularity, and to look to other models; — higher and lower species, large and small animals; primates, and even exotic species.

One of the stated objectives of the conference was to acquaint members of the research community with new or different models, particularly large animal species or primates. This books contains many papers on the physiology, care, surgical approach, behavior, and use of an interesting variety of potential subjects. Many researchers are unaware of the potential that is offered by some of these species. They also appear to be unaware that many volumes have been written and that many conferences like this have been held which have focused on individual animal species.

The laboratory pig, for example, offers researchers an excellent experimental model of the human skin and cardiovascular system. There is voluminous literature available on the pig including a definitive volume, *The Swine in Medical Research*, which was based on a meeting like this and which has a great deal of basic data in it. But similar data also exist in similar volumes compiled on a number of other animals: primate species, bovine and equine species, many of the lower forms, fishes and so forth, even polar bears and giraffes.

The advantages inherent in primates as models of man are obvious, but researchers have been quite slow to capitalize on this particular opportunity. Primates should no longer be looked at as exotic animals that are expensive to acquire, difficult to manage, and about which very little is known. The advent of the original primate research centers which are sponsored by the National Heart Institute has altered that image. A great variety of primates is available in the centers throughout this country. Their cost now compares very favorably with the cost of conditioned laboratory dogs, for example. The means of managing and handling the various primate species have been developed and a superb data base exists on all of the primate species. Beyond that, a primate information center, maintained as a national service, has a computer bibliographic data retrieval system service which is available and will provide practically instantaneous print-outs of the world literature on any primate-related subject. I don't think that is the case for any other experimental animal.

The fact that certain of the primate species have been numbered among the endangered species has been anticipated and breeding colonies have been established. Not only have these proved to be successful enterprises in terms of increasing the absolute numbers of primates available, but they have also attracted the in-

terest of a new group of researchers who are using the availability of the breeding colony to study such things as reproductive biology, mother-infant relationship, developmental abnormalities, sudden infant death syndrome, and many other practical problems with a very obvious relationship to human welfare. The development of primate breeding colonies in conjunction with the primate centers provides the medical research community with a nearly ideal model for study. An animal model closely related to man is now available for study throughout its entire biological range, from conception through geriatrics. The model is a known quantity and its genetics can be predetermined by scientific breeding, its behavior is documented, and, of course, its physical condition can be controlled. The primate center program represents one of the great opportunities for the biomedical research community and I hope that many researchers will look at primate species when designing experiments.

Regardless of the nature of the experiment, the fundamental requirement is to incorporate the selection of an appropriate model as part of the experimental design. Very clearly the selection of an animal model becomes a part of the experimental design. Clearly also, the burden is on the experimenter to justify his selection. I think one could go beyond that and recommend that the scientific journals require justification of the model and its authenticity, perhaps as a part of scientific review.

In all of the foregoing remarks, I have used the cardiovascular system as an area of concern (and the mongrel dog as the whipping boy) primarily because this is the system to which most of the members of this group address themselves. But I think it would require no great imagination to identify other examples of suboptimal models in many other areas of study like atherosclerosis, behavior, cancer research, and so forth.

There is a further opportunity to extend the application of animal models to clinical medicine through deliberate exploitation of the comparative approach. In the comparative approach, one performs what Scholander has called "experiments in nature"; that is, one exploits an exaggerated physiological or pathological characteristic or phenomena exhibited by an animal species as a normal biological variation. One attempts to learn from such experiments certain clues which can be applicable to general processes in animals or man. Relatively few researchers have taken advantage of this opportunity, at least intentionally, but the results of the experiments of comparative physiologists and pathologists have made very major contributions. Let me cite a few examples.

The discovery of the squid giant axon provided membrane investigators with a model for study which has led to significant advances in our understanding of neurophysiology.

The giant muscle cell of the barnacle provides a model which will probably come to equal advantage. We can learn other things from the barnacle as well. The barnacle secretes a cement which will adhere to all manner of surfaces and this would probably be of some interest to bioengineers or even dental researchers.

The secretion of inert gases into the swim bladder of certain fishes is a physiological phenomenon which has obvious basic biological implications.

The peculiar anatomy of the giraffe makes a very interesting model for study of the cerebral circulation. The giraffe's brain is 8 feet or so above its heart and the hydrostatic equivalent of 8 feet is about 200 mm of mercury. So one can postulate that this animal has to have either essential hypertension or a very bad case of cerebral ischemia when he's standing up. Either of these is a very interesting possibility. But the possibilities are even more exciting when the giraffe bends down for a drink, or after drinking, when he raises his head very suddenly. All of these possibilities suggest that some means exists for regulation of the cerebral circulation in the giraffe, and that the anatomical exaggeration peculiar to the giraffe is compensated for by equally exaggerated control systems which then could serve as a model for study.

Some years ago Scholander became concerned over temperature regulation in whales. How does a whale maintain a core temperature of 37° C when its life cycle takes it through the tropics and down into the Antarctic waters where the temperature is around 0° C? It was obvious immediately that the tail fluke served as the radia-

tor, but nevertheless the question remained: How is the core heat conserved? The answer Scholander found was that the veins in the whale form a concentric circle around the arteries. The arteries run centrally and the veins are confluent around them. This is an ideal means for transfer of heat from artery to vein, since the gradient exists from the core out to the periphery. The flow of heat, however, is counter to the flow of blood. This observation formed the basis for the counter-current theory which has come to play an immensely practical role in our understanding of human renal function and human renal disease.

The marine mammals were apparently terrestrial at one point in their evolution and then reverted back to marine existence, but on returning to the marine environment they kept their standard mammalian pulmonary apparatus. Their adaptation has included an ability to dive to 600 feet and to surface without developing the bends. This certainly is a model worth studying. Some can also remain under water for periods of up to 30 or 40 minutes. This latter phenomenon cannot be accounted for on the basis of oxygen storage in the lungs or other places. The secret lies in marine mammals' ability to regulate blood flow to the muscles and peripheral systems. Fundamentally they are able to shut off blood flow to the viscera and to the muscles while they are underwater and keep the flow open to the brain and to the heart. They can, therefore, build up a huge oxygen debt and a huge lactic acid excess. These are superb models, then, for the study of the regional distribution of blood flow.

The comparative analysis of these well-defined animal characteristics can lead to benefits in areas not specifically related to clinical medicine as well. The field of bionics, for example, borrows from the characteristics of animal systems to assist in the design of physical systems or circuits. The rods and cones of the retina, for instance, have been copied as models in the design of receptor circuits. The exquisite sensitivity of the cochlea can respond to physical displacement of a distance less than the diameter of a hydrogen molecule and this has served for models for development of displacement transducers. In neither of these cases can engineers challenge the sensitivity of the biological circuit, but these biological models can serve as ideals to which engineers can aspire.

Animal species have also exhibited a number of well-developed characteristics with which man has only recently become familiar. In a sense, man has only caught up with some of the lower animals. The infrared heat sensors in a snake, the pit viper, can sense the presence of a mouse at a distance of about six feet. Presumably pit vipers have preyed on mice using this technique for eons before man ever thought about developing heat-seeking missiles. Bats used sonar to navigate and to locate their victims long before we invented sonar and, conversely, moths learned to jam the bat's radar before we learned about jamming devices. The list of animals which exhibit physiological or pathological characteristics that could be capitalized on is by no means exhausted. It is only the imagination of the researchers that has been slow in responding.

A related area to which the role of research animals in clinical medicine can be extended is the general development of models of disease. A direct approach to the study of human disease would be the study of man himself, but to a large extent this is not possible.

There is a very practical need to develop analogs of human disease. Many such analogs occur naturally. They represent pathological examples of the "experiments in nature" that I mention. Schmidt-Neilsen, for example, has shown that the sand rat is naturally diabetic, which represents a very interesting model of human diabetes. Scrapies is a neurological disease in the chimpanzee which bears a striking resemblance to some of the demyelinating and sclerosing diseases in man. Investigators have shown that scrapies is transmissable, a major breakthrough in the etiological or epidemiological considerations of neurological diseases.

Disease models are not necessarily limited to mammals. Scarpelli has published a paper in which he has surveyed the spontaneous and experimental disease processes in lower animals and even in invertebrates. He has identified analogs of more than two hundred diseases in a great variety of lower animals, many rarely seen in laboratories. The disease processes

include metabolic, degenerative, neoplastic, and infectious disorders of almost every organ system. Very few of these have been exploited in the laboratory. They remain as unexplained curiosities while the possibility exists that the fundamental processes are identical to human diseases. Since this conference focused to some extent on the cardiovascular system, it is appropriate to point out that there are both opportunities and needs for development of models in the cardiovascular area. Here again opportunities exist for exploitation of the lower forms. Reptiles, for example, have a single ventricle, so that there are hemodynamic parallels between some of the reptiles and children with ventricular septal defects.

Beyond such naturally existing examples, a lot of simple analogs can be easily created. Valve lesions and shunts have been created which parallel to some extent the lesions found in humans, and there are some other far more elegant examples. The need for discovery or creation of models is tremendous. The National Heart Institute, in recent years, has offered substantial rewards in the form of contract support for individuals capable of developing a model of coronary heart disease. I believe it is also fair to say that there is no existing satisfactory animal model of a clinical state which we refer to as congestive heart failure. The variety of animals now used in the study of atherosclerosis suggests that none of those models is entirely valid.

I would like to turn away from models and comment on the need for control and supervision of our research animal stock. I think all of us have read the standard byline which frequently appears in all journals: "Mongrel dogs were anesthetized with Nembutal," and on and on. I think it is possible that at one point in the history of research there was some justification for the use of the semi-starved and anemic, worm-laden pound animal or random rabbit. I think it is also likely that many of the experiments of the past were conducted on animals which were too sickly or rundown to serve as adequate laboratory tools. The recently enacted Animal Research and Animal Welfare legislation provides no real assurance that our animal subjects will, in fact, be healthy or uniform. I think the need for standardization in our animal stocks is all too obvious. This implies that there is a need for genetically controlled sources, where indicated, for adequate nutrition standards, for preliminary clinical studies, and for more appropriate post-mortem studies so the investigator can have some real insight into the validity of both his model and his results.

Finally, I think I would be remiss if I did not express my concern over the absence of sensitivity for the welfare of experimental animals which has been shown by the animal research community. It is a national professional disgrace that two times in our very recent history animal welfare legislation has had to be forced upon us. This legislation was framed for the most part by non-medical and non-research oriented individuals. Few of us provided any real support to the small minority of concerned individuals who tried to protect the interests of the research community. The legislation was introduced by members of the Congress who are not primarily identified with the biomedical research movement, and I think it was supported more out of fear of reprisal by the public than out of real concern and respect for the leadership of the biomedical research community.

Beyond the absence of professional concern and impact on the legislation is the fact that the legislation provided absolutely no funding whatsoever for the major changes which it called for: the renovation of facilities, the implementation of new policies for handling, and so forth, with which we are now forced to conform. One result of this has been an enormous increase in the animal costs at nearly every institution. Across the country institutions have had to dip into other pockets in order to conform to this legislation. It is in the research community's best interests to improve and maintain the welfare of experimental animals and their surroundings, but there is obvious need for inclusion of measures to insure financial support in such legislation as well.

We have only ourselves to blame for this omission because we have abdicated a major responsibility. The long-term implications of continued disinterest and apathy on our part are not palatable and are clearly not in the best

interests of clinical medicine or humanity in general. It is incumbent on us as researchers to assume the leadership which we have failed to acknowledge as individuals. This message was spelled out to us very clearly by the acknowledged spokesman for biomedical research in the Congress, Senator Magnuson, when in speaking at the recent meeting of the AAMC he said,

> "These are questions for which we must find positive answers. Most of these questions are still in your court. Not you alone but you collectively. You are in positions of leadership in these matters. You can exercise that leadership positively or through default pass the ball to others. If you fail to act, then others perhaps less capable than you may design imperfect answers."

In conclusion, I would like to say that we, as researchers, must strive to develop new, more applicable animal models, to use current models to their maximum capacity, and to insure the proper care and handling of all our research animals. We must remember that animal models are a means to study human diseases and that the study of basic physiological parameters of both healthy and diseased states and the methods and techniques of monitoring them requires improved data from research animals.

CHAPTER II

CARDIAC MODELS

Animal Models of Atherosclerosis

Animal Research in Shock

Observations on Experimental Myocardial Infarctions in Dogs

Animal Models of Myocardial Infarction: Current Limitations and Future Promise

Turkey Round Heart Disease: A Model Cardiomyopathy

Studies of a Cardiogenic Shock Model

Biventricular Mechanical Bypass Physiological Studies During Induced Ventricular Failure

Model of Advanced Coronary Atherosclerosis, Myocardial Infarction and Sudden Death with Lethal Arrhythmias in Swine

Experimental Myocardial Infarction in Nonhuman Primates

Canine Coronary Insufficiency Models for Evaluation Systems for Circulatory Support

Pulmonary Artery Constriction in the Cat: A Model for Ventricular Hypertrophy and Congestive Heart Failure

Experimental Myocardial Infarction by Closed Chest Technique

Left Ventricular Failure and Cardiogenic Shock Following Acute Myocardial Infarction: A Correlation of Clinical and Experimental Observations

Proteases and Chemical Mediators in Acute Coronary Occulsion in the Dog

ANIMAL MODELS OF ATHEROSCLEROSIS

Thomas B. Clarkson*

Recent research on the suitability of animal models for research on atherosclerosis has involved numerous studies on nonhuman primates. This interest has developed because of the belief that data obtained from these animals may be more directly related to understanding atherosclerosis of human beings than that obtained from lower animals. Diet-induced atherosclerosis has been studied in certain of the apes and Old World monkeys, particularly chimpanzees *(Pan troglodytes)*, baboons *(Papio species)*, rhesus monkeys *(Macaca mulatta)*, and Cynomolgus macaques *(Macaca fascicularis)*. Among the New World monkey species diet-induced atherosclerosis has been most studied in squirrel monkeys *(Saimiri sciureus)* and cebus monkeys *(Cebus albifrons)*. These nonhuman primates vary considerably in the characteristics of their lipid metabolism and the extent, severity, and morphology of their atherosclerosis. From the available information the investigator can now choose the species best suited for the facet of atherosclerosis that is under study.

INTRODUCTION

The availability of animal models for research on atherosclerosis is particularly important because of the limitation of the kinds of experiments that can be done using human subjects. Until recently progress in research on atherosclerosis was hindered by the lack of satisfactory animal models. Practically all experiments were done on chickens or rabbits, and in some instances the findings had dubious relevance to the human disease. Since the late 1950's there has been a considerable national effort directed toward the characterization of more appropriate animal models. Of particular importance have been studies dealing with the characteristics of naturally-occurring and induced atherosclerosis of nonhuman primates. This recent emphasis on nonhuman primates as animal models of atherosclerosis developed because of the widely held belief that data derived from experiments on these animals may be more directly applicable to man than those obtained from lower animals. The purpose of this communication is to summarize some of the observations on lipid metabolism and atherosclerosis of the nonhuman primate species that are currently of interest in atherosclerosis research.

* Arteriosclerosis Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina

CHIMPANZEE (Pan troglodytes)

Several studies have been done on the natural occurrence of atheroclerosis in chimpanzees.[1-4,7] One of the most extensive of these is that by Strong et al.[4] In that study the aortas of 14 chimpanzees, 21 to 90 months old, were examined for the presence of sudanophilic intimal lesions. Fatty streaks were found in 11 of the 14 aortas; however, no fibrous plaques, complicated, or calcified lesions were present. Vastesaeger and Delcourt[3] reported that two chimpanzees necropsied at the Antwerp Zoo had lesions in the aorta and coronary artery lesions containing foam cells and cholesterol crystals.

The plasma cholesterol concentration of chimpanzees is comparatively low among animals consuming diets natural to the species. Mann[5] fed young chimpanzees for several years purified diets with a small amount of added fruit. With the addition of a small daily intake of 200 mg per day of cholesterol, a hypercholesterolemia of 400 to 500 mg% resulted. The addition of cysteine to the diet, which already contained 20% casein, increased the animals' tolerance for dietary cholesterol. Without added cysteine, Mann states that chimpanzees are about five times more sensitive to dietary cholesterol than are rabbits.

Andrus, Portman and Riopelle[6] studied 14 chimpanzees maintained for one year on semi-purified high fat diets (some of which also contained 1 mg of cholesterol per calorie). With few exceptions their animals were sexually ma-

ture (8 to 24 years). The animals receiving the control diets without added cholesterol had somewhat higher serum cholesterol concentrations when the dietary fat was saturated (coconut oil) than when unsaturated (corn oil). When cholesterol was added to the diet, those receiving coconut fat had a more pronounced hypercholesterolemia. These authors found a high correlation between the mean serum cholesterol concentration and the amount of aortic fatty streaking ($r = 0.70$). Serum cholesterol concentration correlated to a lesser degree with coronary artery lesions and not at all with cerebral artery lesions. Microscopically, the lesions observed in chimpanzees fed the atherogenic diet bore a striking resemblance to early human atherosclerosis, particularly in the cerebral arteries. By comparison with other nonhuman primate models of atherosclerosis, however, these animals are expensive, not plentiful, and their advantage over other nonhuman primates in atherosclerosis research has not been established.

BABOON (Papio species)

Several investigators have studied naturally occurring atherosclerosis of baboons.[8-10] The study by McGill et al.[10] is the most complete of these reports, being based on autopsy studies of 163 baboons trapped in their natural habitat in Kenya, British East Africa. In that study, approximately three-fourths of the adult animals had some degree of aortic fatty streaking after gross staining with Sudan IV; however, no lesions were found in the coronary arteries that resembled early human coronary artery atherosclerosis. Coronary artery intimal lipid accumulation was seen rarely and was not associated with significant reduction in lumen size.

Lipid metabolism of baboons has been studied extensively. A review of some of the current research in this area has been presented by Howard and Kritchevsky.[11] Of particular interest is the fact that plasma cholesterol concentration decreases with age in baboons; whereas, increases with age are seen in most other animals.

To better evaluate baboons as animal models Strong et al.[12] used a factorial design with two levels of added cholesterol (0.5 and 0.01%) fed with diets either high or low in protein and containing either a saturated or unsaturated fat. They concluded that only the level of dietary cholesterol affected the serum cholesterol concentration and this change was extraordinarily small. When the highest level of cholesterol was fed (0.5%), the mean increase for 12 months of study was 12 to 18 mg%. The authors concluded "the magnitude of the increase suggests that the baboon, like man, is only moderately susceptible to dietary cholesterol as measured by serum cholesterol changes." Somewhat higher serum cholesterol concentrations have been reported by Gresham and Howard for baboons fed high butter and egg yolk diets.[13]

The effect of age on cholesterol metabolism of baboons has been studied by Eggen and Strong.[14] Young and old male baboons were used in this study. The fractional contribution of dietary cholesterol was greater in young than in old baboons. The most striking age difference concerned the suppression of endogenous cholesterol synthesis by dietary cholesterol with young baboons having a greater suppression than old animals.

In response to cholesterol-containing diets, baboons developed fatty streaks in the aortic intima, although these lesions were usually small.[15] Coronary artery atherosclerosis is almost never seen even after prolonged periods of feeding dietary cholesterol. It seems unlikely that baboons will become an important primate model for studies on lesions. However, baboons will probably be of particular advantage as animal models for the study of whole body cholesterol metabolism since their ability to absorb cholesterol is about the same as that of man.

RHESUS MONKEYS (Macaca mulatta)

Rhesus monkeys are important animal models of atherosclerosis, particularly for studies on lesions because of the severe and extensive atherosclerosis they develop when fed diets containing added cholesterol. We find that the extent and complexity of diet-induced atherosclerosis in this species exceeds that of any other species of nonhuman primates that we have studied so far and, additionally, one of few in which myocardial infarction occurs with reasonable frequency. They not only develop exten-

sive aortic atherosclerosis but extensive coronary, cerebral, renal and femoral artery atherosclerosis as well. The anatomic distribution of the coronary artery lesions of these animals is another of their advantages; the lesions are more significant in the epicardially distributed proximal branches of the coronary arteries than in the smaller intramyocardial arteries as is the case with many of the other primate models.

Naturally occurring atherosclerosis of rhesus monkeys is uncommon. Chawla et al.[16] studied the prevalence of arterial lesions in 150 rhesus monkeys trapped in India. Among those animals only four had fatty streaks of the aorta and one had atherosclerosis of the aorta. The infrequent occurrence of atherosclerosis among rhesus monkeys fed control diets is in keeping with our own observations and those of others.

A particularly exciting aspect of research on rhesus monkeys as animal models of atherosclerosis has been the finding of type II hyperlipoproteinemia.[17,18] The first of these reports by Greenberg and Moon[18] was that of a single male rhesus monkey. While it appeared that the plasma lipid defect in that monkey was a primary disorder and did fit the type II phenotype, the animal was apparently sterile and the familial character of the disease could not be proven. The report by Morris and Fitch[17] leaves little doubt that the two animals studied by them had primary type II hyperlipoproteinemia. These two animals had plasma cholesterol concentrations averaging 450 to 500 mg%, more than 90% of which was transported by low density lipoprotein, and were not hypertriglyceridemic. Studies are underway in their laboratory to establish if the defect observed in these two monkeys is familial. The intense need for more and better models of primary type II hyperlipoproteinemia suggests the importance of screening the large number of rhesus monkeys being imported into this country yearly.

There has been a considerable advancement in our understanding of lipid metabolism of rhesus monkeys during the past decade. Rhesus monkeys, like several other nonhuman primates, vary in the concentration of free and esterified cholesterol in the plasma not only from day to day but from week to week.[20,21] Alexander and Kopeloff[22] measured the weekly variation in plasma cholesterol concentration in a group of 53 rhesus monkeys and found that the fluctuations tended to disappear when values were recalculated and expressed on the basis of dry serum solids. Their findings indicated that the variability observed was in most cases not due to changes in cholesterol metabolism but rather to changes in the water content of serum.

Another source of variation in plasma cholesterol concentration in rhesus monkeys is the occurrence of pregnancy. Wolf and co-workers[23] recorded the changes in plasma cholesterol concentration of 22 pregnant rhesus monkeys during the course of the pregnancy and for three weeks postpartum. During pregnancy there was more than a 50% decrease in the total plasma cholesterol concentration which occurred by the 12th week and continued throughout pregnancy. After parturition there was a gradual increase and the animals reached control concentration of plasma cholesterol (about 160 mg%) by the third postpartum week.

Rhesus monkeys appear to be useful models for studies on the mechanism of the effects of saturated and unsaturated fat diets on plasma cholesterol homeostasis. Effects of saturated and unsaturated fat diet on most laboratory animals are difficult to demonstrate unless considerable amounts of dietary cholesterol are also included. Emerson and her co-workers[24] have examined the effect of saturated and unsaturated fat diet on lipid metabolism of rhesus monkeys. She and her group demonstrated significant increases in the serum cholesterol concentration of animals fed butterfat and a significant lowering effect due to safflower oil. Further, they showed that small amounts of dietary safflower oil prevented the hypercholesterolemic effect of butter in these animals. As little as 1.8% safflower oil in the diet was capable of preventing the approximate 60 mg% increase due to the addition of 20% butterfat.

Wissler and his co-workers[25] studied the blood lipids of rhesus monkeys fed either "an average American diet" or a "prudent American diet." After 105 weeks, the animals fed the "average American diet" had a mean plasma cholesterol concentration of 358 mg%, while those on the "prudent diet" was about 250

mg%. At autopsy the animals that had been fed the "average diet" had about 75% of their aortic intimal surface involved with pale smooth plaques and fatty streaks, while the animals fed the "prudent diet" had only about 10 to 15% of the surface involved. No reference could be found in their report to the status of the coronary arteries of these animals. This study presents evidence that the serum lipid levels of rhesus monkeys can be increased and aortic atherosclerosis exacerbated when the animals are fed diets physiologic for human beings.

Manning, Clarkson, and Lofland have reported on cholesterol absorption, turnover, and excretion rates of hypercholesterolemic rhesus monkeys.[26] Cholesterol–1,2^{3}H was incorporated into the diets for 195 days and during the isotopic steady state 83 to 92% of the serum cholesterol was derived from dietary cholesterol; thus, 8 to 17% was derived from synthesis. The monkeys consumed about 550 mg of cholesterol per day (from a semi-synthetic diet containing 25% lard and 1 mg/calorie cholesterol) and absorbed from 127 to 192 mg per day. The percentage of fecal total steroids in the form of bile acids varied, ranging from 26 to 47% of the total endogenous cholesterol excreted daily.

Rhesus monkeys have been used to study cholesterol metabolism following surgical ileal bypass.[27] Following ileal bypass there was a decrease in plasma cholesterol concentration that was followed by a return to preoperative concentrations. Experiments were done to measure the rates of hepatic cholesterol biosynthesis and it appeared that the secondary rise in plasma cholesterol concentration was due to increased synthesis of cholesterol in the liver.

It is now apparent that the addition of fat and cholesterol to the diet of rhesus monkeys induces a marked hypercholesterolemia and a severe form of atherosclerosis both in the aorta and coronary arteries. The wide use of rhesus monkeys in atherosclerosis research was no doubt because initial attempts to produce hypercholesterolemia and/or atherosclerosis were not successful. Sperry et al. found that the concentration of serum cholesterol did not change significantly during eight hours after the ingestion of several eggs and that there was only a slight increase after the eggs were fed for several days.[28] When crystalline cholesterol was given orally as an emulsion for four days no rise in the cholesterol concentration of serum occurred. Heuper fed two laboratory-born juvenile rhesus monkeys cholesterol—containing diets.[29] After eight months the animals were examined at necropsy and no atherosclerotic lesions were found. Heuper concluded that rhesus monkeys "do not readily react to a prolonged nutritional intake of excessive amounts of cholesterol with a formation of atheromatous aortic lesion." There is no good explanation for the observation of Heuper.

Taylor and his co-workers pioneered the early studies on diet induced atherosclerosis of rhesus monkeys.[30-33] They reported that the addition of fat and cholesterol to the diet resulted in extensive aortic and coronary artery atherosclerosis as well as cutaneous and tendonous xanthomatosis. Since then their work has been confirmed by numerous investigators.[34,35]

The anatomic distribution and the pathogenesis of rhesus monkey atherosclerosis bears a striking resemblance to that in man. Lesions develop first in the aorta, iliac arteries, and carotid sinus. Coronary artery atherosclerosis follows in its development with the proximal segments of the epicardial coronary arteries developing lesions almost as rapidly as the aorta. When hypercholesterolemia is present for more than 18 months the lesions tend to become larger and more complex and new lesions appear in the peripheral arteries such as the distal coronary intramyocardial branches, mesenteric, spleenic and femoral arteries and the basal circulation of the brain. Complications of lesions such as vascularization, calcification and thrombosis are prominent after 36 months of hypercholesterolemia.

Armstrong and his co-workers[36] have compared the cholesterol content of numerous tissues of normocholesterolemic and hypercholesterolemic rhesus monkeys. The most notable increases in tissue cholesterol concentration were seen in liver, spleen, skin, tendon, muscle and adipose tissue in addition to the increases that might be cardiovascular tissues.

The striking increases in skin and tendon cholesterol content is the result of the remark-

able tendency of rhesus monkey to develop cutaneous and tendonous xanthomatosis.[37] Xanthelasma has been noted in two hypercholesterolemic animals in our colony.

Pickering and his group were the first to study the effect of infant formulas on serum lipid levels of rhesus monkeys.[38] Formulas containing either milk fat or vegetable fat were fed to infant rhesus monkeys for 350 days. The monkeys fed the formula with milk fat had significantly higher serum cholesterol levels (275–404 mg%) than those fed the vegetable fat formula (133–188 mg%). After 350 days of feeding, autopsies were done on the animals. No differences could be found in the frequency of arterial lesions in the two groups of animals.

Greenberg and Gonzalez[39] extended the studies of Pickering and co-workers. They fed infant rhesus monkeys liquid diets that contained either monkey milk, human milk, or one of two prepared formulas that contained either bovine butterfat or a mixed fat with a 1:1 ratio of polyunsaturated to saturated fatty acids. The monkeys were fed the diets for 8–12 months. Arterial lesions were essentially similar in all groups. The monkeys fed human milk had the highest incidence of vascular lesions but the authors concluded that this was probably "a coincidental finding."

Because of the general suitability of diet-induced atherosclerotic lesions of rhesus monkeys, these animals are being used increasingly for studies on lesion regression. Armstrong and his co-workers[40] have reported on an extensive study of lesion regression in rhesus monkeys. In their experiment, forty adult male rhesus monkeys were first fed an atherogenic diet for 17 months and the base line extent of atherosclerosis was determined in a representative sample of ten monkeys. Most had fatty streaks and uncomplicated plaques. The remaining animals were divided into two groups and fed diets containing no cholesterol: one low in fat, the other high in unsaturated fat. After 40 months of being fed these diets the atherosclerosis was again accessed. Stenosis of the lumens of coronary arteries was markedly decreased, as was free and ester cholesterol in the arteries, but a fibrous plaque of considerable size remained. More recently Tucker and his co-workers[41] have studied the regression of diet-induced aortic fatty streaks in rhesus monkeys. The "regression group" in this experiment was fed a cholesterol-containing diet for two months and then normal monkey chow for four months. In all three of the regressed animals studied the intimal smooth muscle cells contained much less lipid than controls. Most of the smooth muscle cells contained no lipid at all and only a very few contained any significant quantities of lipid. In contrast, however, the extracellular lipid appeared more abundant and there was more collagen in the "regressed lesions" than controls.

CYNOMOLGUS MACAQUE (Macaca fascicularis)

Cynomolgus or crab-eating macaques were previously designated as *Macaca irus* and more recently as *Macaca fascicularis*. Although only two groups have reported using these monkeys in atherosclerosis research it seems likely that they will have considerable potential as animal models. Malmros and his co-workers were the first to report on the use of these animals in 1965.[42] They investigated the effect of a hydrogenated coconut oil diet, a corn oil diet, and a diet containing hydrogenated coconut oil and 5% cholesterol on the serum cholesterol concentration of animals of this species. The fats alone appeared to have affected the serum cholesterol concentration similarly with the levels being 100–150 mg%. The addition of 5% cholesterol to the hydrogenated coconut fat diet resulted in an initial increase to about 450 mg% with a gradual plateau to about 300 mg%. On the basis of their observations one would be tempted to conclude that macaques of this species are considerably more resistant to the effects of dietary cholesterol than rhesus monkeys. This appears, however, not to be the case. We have recently initiated experiments in our laboratories in which we are feeding a semisynthetic diet containing 0.5% cholesterol to *Macaca fascicularis* monkeys. In our experience a hypercholesterolemia of 1000 mg% occurred in two months. The striking differences in the hypercholesterolemic response of various species of monkeys to diets that differ from laboratory to laboratory is a little recognized but most important phenomenon.

In a subsequent experiment Kramsch and Hollander fed 12 young *Macaca fascicularis* monkeys a diet containing 1.4% cholesterol by weight. The serum cholesterol concentration of these animals rose above 350 mg% and all the monkeys fed the atherogenic diet developed severe and extensive atherosclerosis of the proximal segments of the coronary arteries. Occlusive lesions of the coronary arteries resulted in the death of two monkeys after 12 months of the diet.[43]

STUMPTAIL MACAQUES (Macaca arctoides)

Stumptail macaques, in the past called *Macaca speciosa,* have been of interest because it is believed by many that they are more docile than other macaques. In our experience it is true that the juvenile animals are remarkably more docile; however, there is very little difference in the tractability of adults as compared with other adult macaques.

A single study has been reported on the susceptibility of these animals to diet-induced atherosclerosis.[44] *Macaca arctoides* fed cholesterol for nine months developed hypercholesterolemia of about 600 mg/100 plasma and had atherosclerotic plaques in the aorta and coronary arteries.

SQUIRREL MONKEYS (Saimiri sciureus)

Squirrel monkeys have been found to offer a number of advantages as animal models of atherosclerosis because they are relatively inexpensive, are easily caged and manipulated, breed well in captivity, are susceptible to naturally-occurring and diet-induced atherosclerosis, and share with man several important aspects of whole body cholesterol metabolism.[45]

The natural occurrence of atherosclerosis has been studied both in wild-caught squirrel monkeys maintained in the laboratory and among free-living animals in the vicinity of Leticia, Colombia, South America. In 1964, Middleton et al.[46] reported on the occurrence of aortic and coronary artery atherosclerosis among wild-caught adult monkeys that had been maintained in the laboratory for periods of three months to one year. About 85% of these animals had fatty streaks of the aorta, visible after Sudan IV staining, while about 7% had small raised plaques primarily in the abdominal aorta that were visible without prior staining. Coronary artery atherosclerosis, although minimal in extent, was present in 30% of these monkeys. Since it was not possible to eliminate the possibility that confinement and diet may have influenced the lesions an additional study was undertaken in which the prevalence and extent of arterial lesions in 220 squirrel monkeys autopsied shortly after being trapped in Colombia, South America, were determined.[47] Fatty streak lesions of the aorta discernible after staining with Sudan IV were infrequent in the juvenile monkeys and were common in the adults with a higher prevalence in the females than in the males. Approximately 10% of the adult monkeys had lipid containing lesions in the small intramyocardial arteries. It was apparent that monkeys surveyed in their natural habitat have lower prevalence of both coronary and aortic atherosclerosis than did monkeys maintained in the laboratory for three to twelve months. It was of interest that the mean serum cholesterol concentration of the free-living monkeys (approximately 100 mg%) was about half that of monkeys maintained in our laboratory and fed low fat cholesterol-free chow.[48]

Because of the differences in serum cholesterol concentration between free-living squirrel monkeys and those in the laboratory it seemed of interest to study the changes in serum cholesterol concentration and lipoprotein cholesterol during the process of capture, confinement, transport, and laboratory acclimation.[49] Blood samples were obtained from a group of adult squirrel monkeys shortly after being trapped and at subsequent intervals during the laboratory acquisition and acclimation process. There were increases in serum cholesterol concentration with each of these processes and increases were noted from the usual 100 mg% noted among free-living animals to concentrations of about 200 mg% in the laboratory. Investigators using these animals, especially in diet or drug studies, should recognize that a significant hypercholesterolemia may exist at the start of the experiment.

Atherosclerosis can be markedly exacerbated by the addition to the diet of fat and

cholesterol.[50,51] Diet exacerbated lesions in squirrel monkeys are somewhat less extensive and complex than diet-induced lesions of rhesus monkeys. Most commonly, the aortas from cholesterol-fed squirrel monkeys will have extensive fatty streaking covering almost the entire intimal surface with numerous small plaques about 2 or 3 mm in diameter affecting about 40% of the thoracic aorta and about 20% of the abdominal aorta. Coronary artery lesions are small and infrequent in the proximal main branches but common and extensive in the small intramyocardial branches.

Among squirrel monkeys fed cholesterol, certain individuals (hyperresponders) develop severe hypercholesterolemia while others (hyporesponders) fed the same diet maintained plasma cholesterol concentrations near that of controls. The result of recent experiments suggests that these differences are genetically determined.[52] Hypo- and hyperresponder squirrel monkeys, by selective breeding, have been used to develop strains of squirrel monkeys with these metabolic characteristics. Hyporesponder squirrel monkeys when fed cholesterol-containing diets even for a long period have only minimal arterial lesions. In contrast, however, the atherosclerosis of hyperresponder monkeys is markedly exacerbated. The lesions seen in hyperresponder monkeys are essentially the same as those that we have previously reported[50] except that they are more extensive and severe and lesions frequently associated with hyperlipidemia are seen in other organs. About half of hyperresponder monkeys have cutaneous xanthomatosis and an occasional animal will have xanthelasma. When xanthomtosis occurs it is most often present on the planter or palmer surfaces.

Using formula diets and sterol balance techniques cholesterol absorption, synthesis, excretion, and turnover have been compared in hypo- and hyperresponding monkeys. Cholesterol absorption, synthesis and excretion of neutral steroid were similar for hypo-and hyperresponders; however, hyporesponders were found to have increased bile acid excretion shortly after cholesterol feeding was begun, which was present among hyperresponders to a much lesser degree. These experiments have suggested that the mechanism of control of plasma cholesterol concentrations in cholesterol-fed squirrel monkeys is related to the rate of conversion of cholesterol to bile acids.[53]

Squirrel monkeys have also been found to be useful animal models for studies on the contribution of the intestinal wall to the biosynthetic origin of serum cholesterol[54] and for studies on the effect of dietary cholesterol on the relative rates of synthesis in various tissues.[55] Additionally, the usefulness of these animals has been shown for studies on the effect of dietary fat on whole body sterol metabolism.[56]

Atherosclerotic aortas have been studied metabolically by St. Clair and co-workers.[57] The changes in lipid metabolism of the diseased aortas resemble those reported as occurring in other species. Among the fatty acids esterified to cholesterol the amount of cholesteryl oleoate increased the most. Additionally, synthesized fatty acids incorporated into cholesteryl esters were increased approximately six-fold in the atherosclerotic aortas compared to controls.

Squirrel monkeys have been used to study the effect of carbon monoxide on the extent and severity of atherosclerosis.[58] In that seven month study the atherosclerosis induced in the coronary arteries was aggravated by carbon monoxide but aortic atherosclerosis was not. Among the monkeys exposed to carbon monoxide there was more intimal lipid in each coronary artery atherosclerotic plaque rather than more coronary arteries being affected with atheosclerosis.

Lehner and co-workers[59] reported on the effect of insulin deficiency, hypothyroidism, and hypertension on diet exacerbated atherosclerosis of squirrel monkeys. Insulin-deficient, hypothyroid, and hypertensive monkeys were found to have more extensive coronary artery and aortic atherosclerosis than did controls. Atherosclerosis was especially severe in the insulin-deficient monkeys. This study suggested that the squirrel monkey may be a good animal in which to study the mechanisms by which these disorders affect atherosclerosis.

CEBUS MONKEYS (Cebus albifrons)

Cebus albifrons monkeys are among the species in which the earliest studies were done on

diet-induced atherosclerosis in nonhuman primates.[60,61] These animals are particularly interesting as animal models of atherosclerosis because of a striking age and sex difference in their susceptibility to diet-induced atherosclerosis.[62-64] Immature cebus monkeys fed an atherogenic diet (25% lard, 0.5% cholesterol) maintain lower concentrations of serum cholesterol than adults fed the same diet and adult male monkeys have markedly higher concentrations than adult females.

Adult but not young *Cebus albifrons* monkeys are moderately susceptible to diet-induced atherosclerosis. The atherosclerosis of these monkeys is characterized by slight lesions in the aorta, relatively extensive lesions in the coronary arteries, large raised plaques of the carotid bifurcation, the absence of lesions in the cerebral vessels, and the presence of lesions in the small intramuscular arteries of the tongue, uterus, and kidneys.

SUMMARY

Some of the characteristics of lipid metabolism and atherosclerosis of the most commonly used nonhuman primates in atherosclerosis research have been presented. The availability of these more nearly suitable animal models is one of the most important advances in the field of atherosclerosis during the past decade. While much work remains to be done to fully characterize the existing models, an investigator can now choose the animal model most logical for use in studies of particular facets of the disease: cholesterol absorption, cholesterol excretion, aortic atherosclerosis, coronary artery atherosclerosis, cerebral artery atherosclerosis or peripheral arterial disease.

ACKNOWLEDGEMENTS

The helpful advice of my colleagues Drs. B. C. Bullock, N. D. M. Lehner, H. B. Lofland, R. W. Prichard, and R. W. St. Clair is gratefully acknowledged.

Supported in part by a National Institutes of Health Grant HE14164-01.

REFERENCES

1. Fox, H. Some comments on atherosclerosis in wild mammals and birds. N. Y. Acad. Med. Bull. 15:748-756, 1939.
2. Stare, F. J., Andrus, S. B., and Portman, O. W. "Primates in Medical Research with Special Reference to New World Monkeys," in D. E. Pickering (ed.): *Proceedings of Conference on Research with Primates*, Beaverton, Oregon: Tektronix Foundation, 1958, pp. 59-66.
3. Vastesaeger, M. M., and Delcourt, R. Spontaneous atherosclerosis and diet in captive animals. Nutr. Diet. 3:174-188, 1961.
4. Strong, J. P., Kritchevsky, D., and Martinex, R. D. Sudanophilic aortic lesions in chimpanzees. Arch. Path. 81:544-547, 1966.
5. Mann, G. V. Diet and cholesterolemia in chimpanzees. Fed. Proc. 22:268, 1963.
6. Andrus, S. B., Portman, O. W., and Riopelle, A. J. Comparative studies of spontaneous and experimental atherosclerosis in primates. II. Lesions in chimpanzees including myocardial infarction and cerebral aneurysms. Progr. Biochem. Pharmacol. 4:393-419, 1968.
7. Stout, C., and Lemmon, W. B. Predominant coronary and cerebral atherosclerosis in captive nonhuman primates. Exp. Molec. Path. 10:312-322, 1969.
8. Lindsay, S., and Chaikoff, I. L. Arteriosclerosis in the baboon. Arch. Path. 63:460, 1957.
9. Gillman, J., and Gilbert, C. Atherosclerosis in the baboon *(Papio ursinus)* its pathogenesis and etiology. Exp. Med. and Surg. 15:181, 1957.
10. McGill, H. C., Strong, J. P., Holman, R. L., and Werthessen, N. T. Arterial lesions in the Kenya baboon. Circ. Res. VIII:670-679, 1960.
11. Howard, A. N., and Kritchevsky, D. I. Lipid metabolism in the Baboon. Baboon in medical research. First International Symposium on the Baboon and Its Use as an Experimental Animal. San Antonio—1963.
12. Strong, J. P., Rosal, J., Deupree, R. H., and McGill, H. C., Jr. Diet and serum cholesterol levels in baboons. Exp. Molec. Path. 5:82-91, 1966.
13. Gresham, G. A., and Howard, A. N. Vascular lesions in primates. Ann. N. Y. Acad. Sci. 127:694, 1965.
14. Eggen, D. A., and Strong, J. P. Diet and cholesterol metabolism in young and old baboons. Atheroscler. 12:359-369, 1970.
15. Strong, J. P., and McGill, H. C., Jr. Diet and experimental atherosclerosis in baboons. Am. J. Path. 50:669-690, 1967.
16. Chawla, K. K., Murthy, C. D. S., Chakravarti, R. N., and Chhuttani, P. N. Arteriosclerosis and thrombosis in wild rhesus monkeys. Am. Heart J. 73, 1:85-91, 1967.
17. Morris, M. D., and Fitch, C. C. Spontaneous hyperbetalipoproteinemia in the rhesus monkey. Biochem. Med. 2:209-215, 1968.
18. Greenberg, L. D., and Moon, H. D. Blood lipid studies in a rhesus monkey with essential hypercholesterolemia. Fed. Proc. 20:256, 1961.

19. Greenberg, L. D. Plasma cholesterol levels of monkeys maintained on various controlled and deficient diets. Am. J. Clin. Nutr. 23, 8:1101–1104, 1970.
20. Alexander, G. J., Koeploff, L. M., and Taylor, R. M. Hypocholesteremic effect of pentamethylenetetrazol (Metrazol) in the monkey *M. mulatta.* Arch. Intern. Pharmacodyn. 163, 1966.
21. Kritchevsky, D., and McCandless, R. F. J. Weekly variations in serum cholesterol levels of monkeys. Proc. Soc. Exp. Biol. Med. 95:152, 1957.
22. Alexander, G. J., and Kopeloff, L. M. Fluctuations in serum cholesterol levels in the monkey. Am. J. Med. Sci. 257:24–31, 1969.
23. Wolf, R. C., Temte, L., and Meyer, R. K. Plasma cholesterol in pregnant rhesus monkeys. Soc. Exp. Biol. & Med. 125:1230-1231, 1967.
24. Emerson, B. A., Walker, J. B., Ganapathy, S. N. Effects of saturated and unsaturated fats and their mixtures on the lipid metabolism of monkeys. J. Nutrition 76:6, 1962.
25. Wissler, R. W., Hughes, R. H., Frazier, L. E., Getz, G. S., and Turner, D. Aortic lesions and blood lipids in rhesus monkeys fed "table prepared" human diets. Circ. 32: 220, 1965.
26. Manning, P. J., Clarkson, T. B., and Lofland, H. B. Cholesterol absorption, turnover, and excretion rates in hypercholesterolemic rhesus monkeys. Exp. Molec. Path. 14:75–89, 1971.
27. Moutafis, C. D., and Myant, N. B. Increased hepatic synthesis of cholesterol after ileal bypass in monkeys. Clin. Sci. 34:541–548, 1968.
28. Sperry, W. M., Jailer, J. W., and Engle, E. T. The influence of diet on the cholesterol concentration of the blood serum in normal, spayed and hypothyroid monkeys. Endocrin. 35:38–48, 1944.
29. Heuper, W. C. Experimental studies in cardiovascular pathology. XIV Experimental atheromatosis in macacus rhesus monkeys. Am. J. Path. 22:1287–1291, 1946.
30. Taylor, C. B., Cox, G. E., Manalo-strella, P., Southworth, J., Patton, D. E., and Cathcart, C. Atherosclerosis in rhesus monkeys: II. Arterial lesions associated with hypercholesterolemia induced by dietary fat and cholesterol. Arch. Path. 74:16, 1962.
31. Taylor, C. B., Trueheart, R. E., and Cox, G. E. Atherosclerosis in rhesus monkeys: III. The role of increased thickness of arterial walls in atherogenesis. Arch. Path. 76:14, 1963.
32. Taylor, C. B., Manalo-Estrella, P., and Cox, G. E. Atherosclerosis in rhesus monkeys: V. Marked diet-induced hypercholesteremia with Xanthomatosis and severe atherosclerosis. Arch. Path. 76:239, 1963.
33. Taylor, C. B., Patton, D. E., and Cox, G. E. Atherosclerosis in rhesus monkeys: VI. Fatal myocardial infarction in a monkey fed fat and cholesterol. Arch. Path. 76:404, 1963.
34. Wissler, R. W., Getz, G. X., Vesselinovitch, D., Frazier, L. E., Hughes, R. H. Acute severe experimental atherosclerosis in rhesus monkeys. Fed. Proc. 25:597, 1966.
35. Scott, R. F., Morrison, E. S., Jarmolych, J., Nam, S. C., Kroms, M., and Coulstron, F. Experimental atherosclerosis in rhesus monkeys. I. Gross and microscopic features and lipid values in serum and aorta. Exp. Molec. Path. 7:11–33, 1967.
36. Armstrong, M. L., Connor, W. E., and Warner, E. D. Xanthomatosis in rhesus monkeys fed a hypercholesterolemic diet. Arch. Path. 84:227–237, 1967.
37. Armstrong, M L., Connor, W. E., and Warner, E. D. Tissue cholesterol concentration in the hypercholesterolemic rhesus monkeys. Arch. Path. 87:87–92, 1969.
38. Pickering, D. E., Fisher, D. A., Perley, A., Basinger, G. M., and Moon, H. D. Influence of dietary fatty acids on serum lipids; studies of the immature rhesus monkeys *(Macaca mulatta).* Am. J. Dis. Child. 102:42–51, 1961.
39. Greenberg, L. D., and Gonzalez, I. E. Influence of fatty acid composition of infant formulas on development of arteriosclerosis. Exp. Molec. Path. 10:240–249, 1969.
40. Armstrong, M. L., Warner, E. D., and Conner, W. E. Regression of coronary atheromatosis in rhesus monkeys. Circ. Res. 57:59–67, 1970.
41. Tucker, C. F., Catsulis, C., Strong, J. P., and Eggen, D. A. Regression of early cholesterol-induced aortic lesions in rhesus monkeys. Am. J. Path. 65, 3:493–502, 1971.
42. Malmros, H., Wigand, G., Kockum, I. Experimental hypercholesterolemia and hypertriglyceridemia in cynamolgus monkeys fed saturated fat and cholesterol. J. Atheroscler. Res. 5:474, 1965.
43. Kramsch, Dieter M., and Hollander, W. Occlusive atherosclerotic disease of the coronary arteries in monkey *(Macaca irus)* induced by diet. Exp. Molec. Path. 9:1–22, 1968.
44. Pick, R., and Katz, L. N. Cholesterol-fat induced atherosclerosis in the stumptail macaque *(Macaca speciosa).* Supplement II to Circulation 39:III–20, 1969.
45. Clarkson, T. B., Bullock, B. C., Lehner, N. D. M., and Feldner, M. A. The squirrel monkey as a laboratory animal. Natl. Acad. Sci., Natl. Res. Council Pub. 1954, 1968.
46. Middleton, C. C., Clarkson, T. B., Lofland, H. B., and Prichard, R. W. Atherosclerosis in the squirrel monkey. Naturally-occurring lesions of the aorta and coronary arteries. Arch. Path. 78:16–23, 1964.
47. Middleton, C. C., Rosal, J., Clarkson, T. B., Newman, W. P., and McGill, H. C., Jr. Arterial lesions in squirrel monkeys. Arch. Path. 83:352–358, 1967.
48. Lofland, H. B., St. Clair, R. W., MacNintch, J. E., and Prichard, R. W. Atherosclerosis in New World primates. Arch. Path. 83: 211, 1967.

49. St. Clair, R. W., MacNintch, J. E., Middleton, C. C., Clarkson, T. B., and Lofland, H. B. Changes in serum cholesterol levels of squirrel monkeys during importation and acclimation. Lab. Invest. 16:828–832, 1967.
50. Middleton, C. C., Clarkson, T. B., Lofland, H. B., and Prichard, R. W. Diet and atherosclerosis of squirrel monkeys. Arch. Path. 83:145–153, 1967.
51. Maruffo, C. A. and Portman, O. W. Nutritional control of coronary artery atherosclerosis in the squirrel monkey. J. Atheroscler. Res. 8:237–247, 1968.
52. Clarkson, T. B., Lofland, H. B., Bullock, B. C., and Goodman, H. O. Genetic control of plasma cholesterol. Arch. Path. 92:37–45, 1971.
53. Lofland, H. B., Clarkson, T. B., St. Clair, R. W., and Lehner, N. D. M. Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes. J. Lipid Res. 1971. (In Press)
54. Dietschy, J. M., and Wilson, J. D. Cholesterol synthesis in the squirrel monkey: relative rates of synthesis in various tissues and mechanisms of control. J. Clin. Invest. 47:166–174, 1968.
55. Wilson, J. D. Biosynthetic origin of serum cholesterol in the squirrel monkey: evidence for a contribution by the intestinal wall. J. Clin. Invest. 47:175–187, 1968.
56. Lofland, H. B., Clarkson, T. B., and Bullock, B. C. Whole body sterol metabolism in squirrel monkeys *(Saimiri sciureus)*. Exp. Molec. Path. 13:1–11, 1970.
57. St. Clair, R. W., Lofland, H. B., and Clarkson, T. B. Influence of atherosclerosis on the composition, synthesis, and esterification of lipids in aortas of squirrel monkeys *(Saimiri sciureus)* J. Atheroscler. Res. 10:193–206, 1969.
58. Webster, W. S., Clarkson, T. B., and Lofland, H. B. Carbon monoxide-aggravated atherosclerosis in the squirrel monkey. Exp. Molec. Path. 13:36–50, 1968.
59. Lehner, N. D. M., Clarkson, T. B., and Lofland, H. B. The effect of insulin deficiency, hypothyroidism, and hypertension on atherosclerosis in the squirrel monkey. Exp. Molec. Path. 15:230–244, 1971.
60. Mann, G. V., Andrus, S. B., McNally, A., and Stare, F. J. Experimental atherosclerosis in cebus monkeys. J. Exp. Med. 98:195, 1953.
61. Wissler, R. W., Frazier, L. E., Hughes, R. H., and Rasmussen, R. A. Atherogenesis in the Cebus monkey. I. A comparison of three food fats under controlled dietary conditions. Arch. Path. 74:312, 1962.
62. Lofland, H. B., Clarkson, T. B., St. Clair, R. W., Lehner, N. D. M., and Bullock, B. C. Atherosclerosis in *Cebus albifrons* monkeys. I. Sterol Metabolism. Exp. Molec. Path. 8:302–313, 1968.
63. Lofland, H. B., Jr., St. Clair, R. W., Clarkson, T. B., Bullock, B. C., and Lehner, N. D. M. Atherosclerosis in Cebus monkeys. II. Arterial Metabolism. Exp. Molec. Path. 9:57–70, 1968.
64. Bullock, B. C., Clarkson, T. B., Lehner, N. D. M., Lofland, H. B., Jr., and St. Clair, R. W. Atherosclerosis in *Cebus albifrons* monkeys. III. Clinical and Pathologic Studies. Exp. Molec. Path. 10:39–60, 1969.

DISCUSSION

Chairman Kezdi: Thank you Dr. Clarkson.

C. Grant La Farge, Children's Hospital Medical Center, Boston, Mass.: The non-human primates have always been very appealing to everybody because they are readily available and cheap in spite of the fact that they are not very consistent. While I grant you that the perfect model is worth any price, can you give us an idea of how expensive they are?

T. B. Clarkson: Yes, I can give approximate figures. Squirrel monkeys cost about $40 to $45, cebas monkeys about $45 to $50, and adult size rhesus monkeys perhaps $75 to $80.

D. M. Kramsch, Boston University: (Question on cholesterol in milk.)

T. B. Clarkson: You mean: have we examined whether the amount of cholesterol in the milk makes any difference? Well, this study is underway at the moment. I don't feel that I'm clear enough on the results to make a comment on that matter. We do have some hand-reared animals with and without cholesterol. We should be able to piece that together fairly soon.

P. Somani, Abbott Laboratories, North Chicago: I was struck by the slide of the heart where the coronary arteries were very well occluded and yet the myocardium seemed normal. Is that peculiar to rhesus monkey, or what is the reason for this? Do you have some explanation?

T. B. Clarkson: Yes; my explanation is that slides can be deceptive if you show them fast enough. These arteries are deceptive insofar as the lipid which you see is correlated with the adventitial accumulation of lipids. The lumens are not as stenosed as you would think they might be from the gross photograph. They're more often of the nature that I showed you in the histologic section, with about 75% occlusion of the lumen. In effect, a fixed preparation. But I think these gross photographs of Macacs are deceptive because of the amount of adventitial lipids. I expect that Dr. Kramsch would agree.

ANIMAL RESEARCH IN SHOCK

R. M. Hardaway, III*

With exception of clinical observations made during World War II and the Korean conflict, essentially all shock research prior to 1964 was carried out utilizing animals. This was necessitated by the fact that acute, severe injuries in civilian practice occur at irregular and infrequent intervals not conducive to organized study, and because of the resources required merely to treat the patient, much less to study him. Beginning in 1964 several clinical shock study units were organized, and considerable research was done utilizing clinical patients. Animal studies continued and greater use was made of primates.

Much fundamental work was accomplished utilizing dogs, and the bulk of the information on shock has resulted therefrom. The work encompassed hemodynamic effects, hormones, cardiac, respiratory, and blood coagulation studies, bacterial toxins, hemorrhage, trauma, etc. The role of the various treatments including intravenous fluids and drugs were explored primarily with animals. Great care must be taken in interpolating between animals and man, however, and some misleading conclusions were made. Brief summaries are made of studies in pathophysiology and treatment of shock in animals which resulted in important new concepts in the treatment of clinical shock.

INTRODUCTION

The first scientific investigations in the field of shock were made in 1890 by Crile[1] utilizing animals. Important scientific observations in the field of shock in humans were made by U.S. Army medical officers in World War I, and several hypotheses developed. Following World War I, however, research in shock was essentially confined to work with animals. This was primarily the result of the following conditions:

1. The occurrence of clinical shock in humans was relatively uncommon and unpredictable in civilian medical practice.
2. When clinical shock did occur, the entire capabilities and resources of the treatment center were devoted to treatment only, leaving none for research.
3. Clinical research was more difficult than laboratory research. Adequate controls were nearly impossible, whereas in laboratory research most variables could be controlled.
4. The cost of animal research was small compared with that involved in clinical research. Very little money was available for any type of research until after World War II.

METHOD

The animals used in shock research were numerous. Possibly the most important and commonly used animal was the dog. The dog had a number of advantages: it was a relatively large animal with a large blood volume and more or less human-sized vessels and organs, and its anatomy and behavior were amenable to laboratory conditions. A classic hemorrhagic shock model, utilizing the dog, was developed by Wiggers,[2] which, together with its modifications, has become a standard in the field. The dog also has been used to study hemodynamic alterations, concepts of irreversibility, influence of steroids, and other aspects of shock.

Another animal frequently used in shock studies was the rat, which had the advantage of being inexpensive and available in great numbers. The rat was used most commonly in studies of traumatic shock, hemorrhagic shock, concepts of host resistance and tolerance, RES blockade, microcirculatory changes, and biochemical shifts in the blood. More recently with the development of fine surgical techniques, the rat has become particularly adapted for work with hemorrhagic shock.

The rabbit has been used primarily for work with endotoxin, generalized Shwartzman reaction, and other toxins.

*Brigadier General, Medical Corps Commanding General, William Beaumont General Hospital, El Paso, Texas 79920

The cat—primarily a favorite of the neurological related researchers—was used mostly in studies of shock in the nervous system and, to some extent, histamine research.

The mouse was used primarily for work with anaphylactic shock and burns. The mouse is inexpensive, but its small size makes surgical procedures difficult.

In recent years, primates have come into general use in many types of shock research by virtue of their phylogenic relationship to man. They have the disadvantages of being frequently disease-ridden, particularly with tuberculosis; they are apt to be mean and difficult; their vessels are friable; and their anatomy is relatively small.

The major problem with animal research in shock is that all animals differ in various respects from man. For instance, the dog has an unusually receptive gastrointestinal tract to changes in shock, and the rabbit is particularly susceptible to bacterial toxins. One of the most important, but perhaps least recognized difference, is the coagulability differences between man and dog. Dog blood clots more easily than human blood. This has been the occasion for misunderstandings, particularly in the field of open heart surgery, as well as in the field of shock research. In spite of these differences, most of the advances made in study of shock have been accomplished on animals—most prominently in the dog.

One of the most interesting and definitive studies carried out in dogs involves the disseminated intravascular coagulation and its relationship with shock of various types. With regard to hemorrhagic shock, the first observation on hypercoagulability and clotting, as contributed to by acute hemorrhage, was performed on humans by Hewson [3] in the early 18th century. In the process of producing severe hemorrhage during the therapeutic procedure of blood letting, he noted that that which was taken away last was first coagulated. In other words, hemorrhage produced a hastening of coagulation.

Definitive studies of this phenomena were started at Walter Reed Army Institute of Research in 1960.[4] The decrease in clotting time of the blood after severe hemorrhage was correlated with the onset of a severe acidosis due to lactiacidemia secondary to cellular anoxia. This may or may not be due to the inactivation of endogenous heparin by acidosis (Figure 1). It was found that severe hemorrhage reduced the clotting time in dogs to a small fraction of the normal. It was also found that if the shock thus produced by the hemorrhage was prolonged, the clotting time would suddenly become indefinitely prolonged and the blood become completely incoagulable (Figure 2). It was also shown that if the blood became incoagulable the animals would die even if all of their blood were immediately returned. If this phenomena did not occur, the dogs would survive.

These experiments were done using the Wiggers reservoir system, although it was necessary to avoid the use of heparin used by Wiggers in order to study the coagulation mechanism. The blood was kept fluid by means of an ion absorbing resin column. Wiggers [5] and many others have shown that dogs invariably die if their blood pressure is reduced to approximately 40 mm of mercury over a period of 1–3 hours. This conception has continued down to the present time. However, at Walter Reed, experiments in 1962 [6] showed that this phenomena of irreversibility was not associated in any way with the intensity or the duration of the hemorrhagic shock period, but in fact was associated with the use of the Wiggers reservoir. While in the reservoir the blood in some way became toxic to a dog in hemorrhagic shock even though it was his own blood. Investigations to explain this mystery showed that if one substituted a polyethylene bag, for the open reservoir, eliminated all contacts of the extracorporeal blood with air, metal, glass, or any substance with the exception of the plastic, and avoided the flow of blood over the plastic more than one time, dogs could survive for periods up to 24 hours in hemorrhagic shock with 35 mm of mercury with a very low mortality rate (Figure 3). The hypercoagulable circulating blood in the animal did not clot in the circulation unless clotting was initiated by some other factor. This could be by the contact of the blood with a foreign surface, including air, glass, metal, etc. If the extracorporeal blood was altered, the hypercoagulable stagnant flow in the animal resulted in disseminated intravascular coagulation with

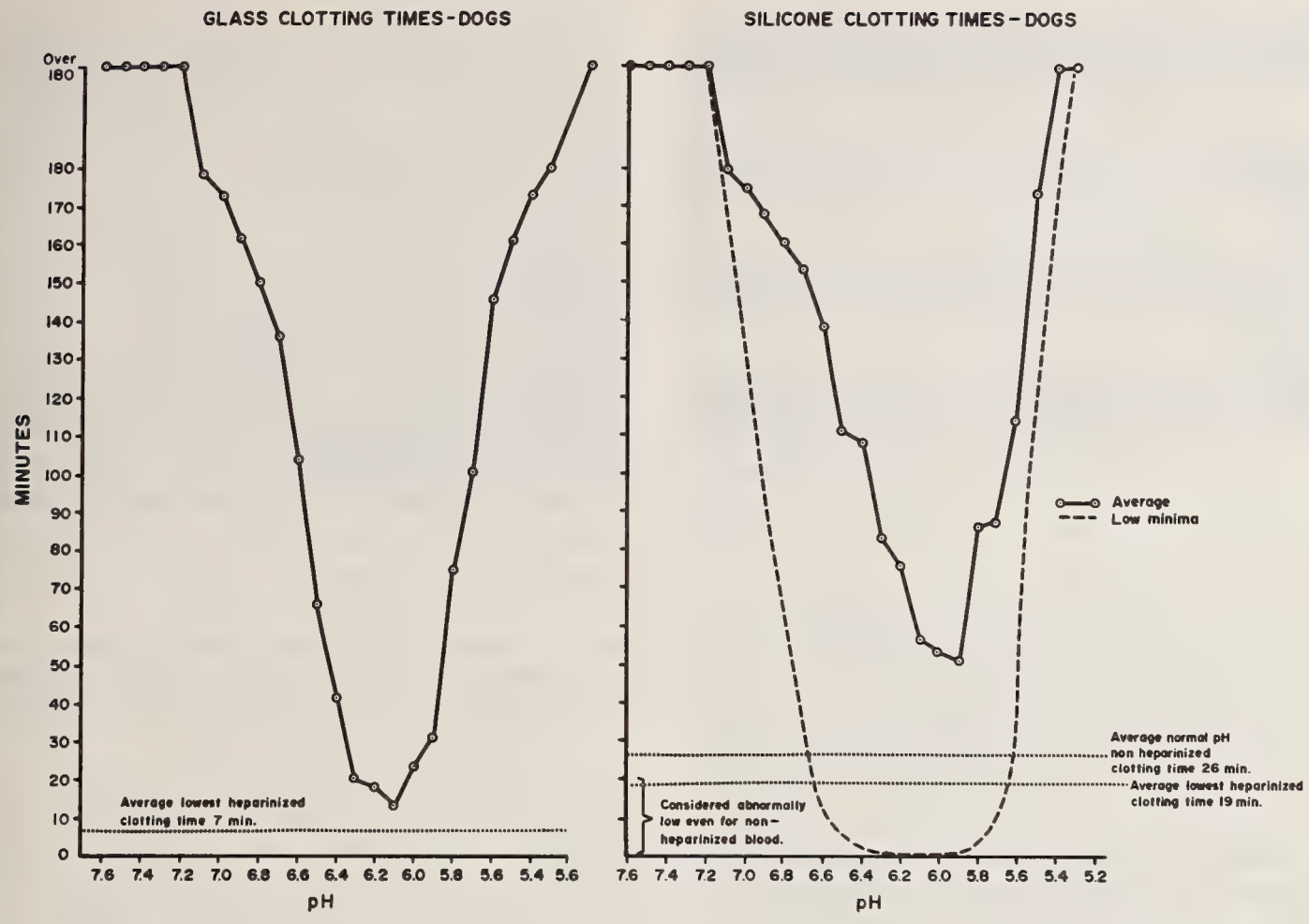


FIGURE 1.—Glass and silicone clotting times of dog's blood at different pH's. Average values for each pH do not represent the average shortest clotting time because some clotting times are going up while others are going down. The average lowest clotting time reached at any pH is indicated, as is the minimal silicone clotting time at each pH. Note that the average lowest clotting time of heparinized blood is shorter than average clotting time for nonheparinized blood at normal pH's. Similar shortening takes place in nonheparinized blood but the initial time is so short it has less room to fall.

the onset of a consumptive coagulapathy and micro-infarction of vital organs and death. It was also shown that this same phenomena took place in open heart surgery. This accounted for the poor results in experiments using dogs in open heart surgery before clinical use of open heart procedures were initiated. It was also found that while this same phenomena occurred in humans, that the lessened coagulability of blood in humans made it less common—occurring only in the presence of shock. This difference between dogs and humans held back open heart surgery several years because it was thought that humans would react as did the dog and die in a short time on the pump. Actually, humans exhibit the same phenomena but it requires a longer time on the pump, so that open heart surgery is feasible.

The conclusion of the above experiments was that the occurrence of DIC required two separate initiating factors: (1) a slow flowing, acid, hypercoagulable blood as produced by shock, and (2) a thromboplastic agent of some type. The first factor without the second would not result in DIC. Therefore, hemorrhagic shock alone was innocuous and reversible up to the point of death. However, many factors could contribute a thromboplastic agent into the blood stream during shock. A series of investigations[7] was carried out in order to define some of these factors. They were found to be: (1) bacterial toxins, either indotoxin or endotoxin, (2) hemolysis even in minute amounts, (3) tissue trauma or crushing, and (4) other miscellaneous factors, including cellular debris (amniotic fluid), cancer, and viruses.

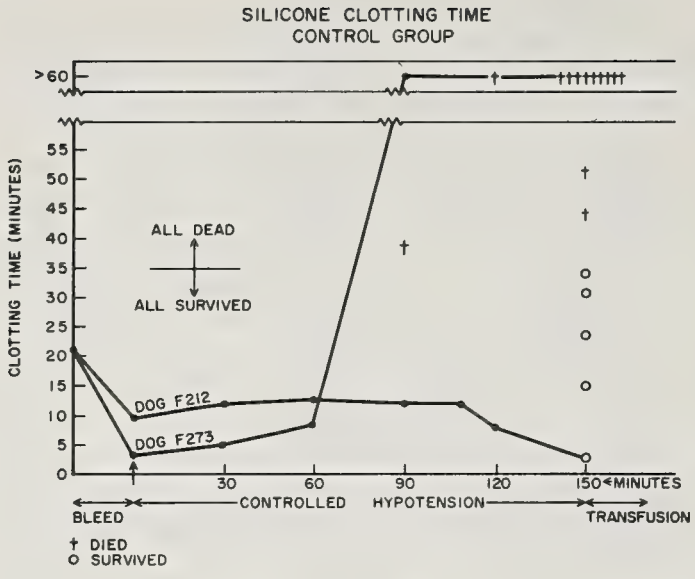


FIGURE 2.—Silicone clotting times of dogs subjected to hemorrhagic shock. Bleeding produced a decrease in clotting time. Those dogs that later showed a prolonged clotting time (over 35 minutes) died (as noted by the crosses); those which did not, survived (as noted by the circles). Two typical animals are shown by the lines.

These factors were subsequently investigated in a series of experiments.[8] I'll describe tissue trauma and hemolysis first. It was found that if a dog was anesthetized and mallet trauma applied to one thigh, and the animal then subjected to an otherwise nonfatal hemorrhagic shock, it would survive. However, if 48 hours were allowed to elapse between the trauma and the hemorrhage the animal would die. This phenomena was seen to be correlated with the appearance of a hemoglobin or hemoglobin-like pigment which appeared in the blood stream with a peak level of 48 hours, post-trauma (Figure 4). It was, therefore, decided to see the effect of a minute amount of hemolysis on the non-fatal hemorrhagic shock. Accordingly, 20 cc of the animal's own blood was withdrawn, hemolized by freezing, and returned to the animal. This produced a level of hemoglobin in the blood approximately equal to the trauma 48 hours after it was applied. Immediately following the administration of the animal's own hemolized blood, the dog was subjected to a nonfatal hemorrhagic shock. The animal died with the coincidental production of an incoagulability. At autopsy, it was found to have suffered the typical changes of DIC including focal infarction. In addition to these studies, it was found that this fatal DIC could be prevented either by pre-treatment with heparin or by treating the animal during shock with fibrinolysin, which apparently dissolved the intravascular clots (Figure 5).[9]

Studies on the effect of bacterial toxins[10] showed that all types of toxins produced changes typical of DIC. Even a minute dose of E. coli indotoxin produced an immediate incoagulapathy of the blood with the immediate disappearance of platelets. A fall in fibrinogen and other clotting factors increased over a period of a few hours and produced death. It was found that this phenomena could not be prevented with heparin which was somewhat surprising, and several studies involving the use of heparin were initiated.[11] It was found that heparin had no influence on the aggregation of platelets, which was the first phenomena

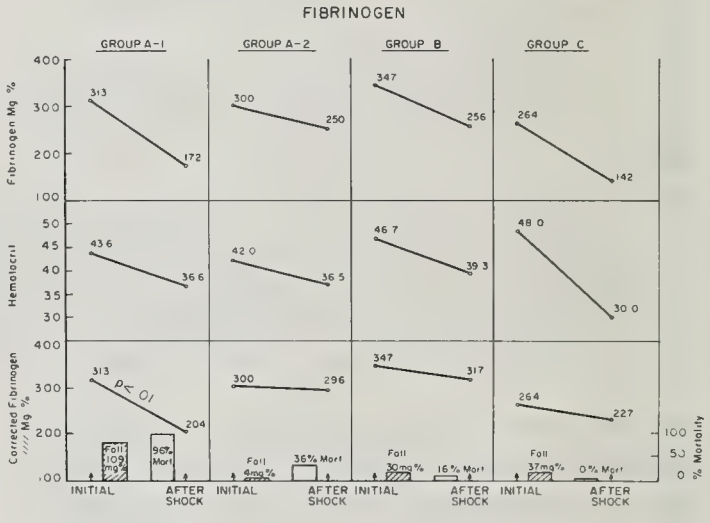


FIGURE 3.—Fibrinogens of groups of dogs subjected to hemorrhagic shock. Group A1 was subjected to hemorrhagic shock by the Wiggers reservoir technique, with a blood pressure of 40 for four hours. Fibrinogen fell from 313 mg% to 172 mg%. The corrected fibrinogen levels were calculated using the hematocrit as a measure of dilution. This left a significant fibrinogen fall. This utilization of fibrinogen was correlated with a high mortality rate (96%). Group A2 was treated similarly but heparinized. This prevented mortality and fibrinogen fall. Group B did not use the Wiggers reservoir but a plastic bag with prevention of exposure of blood to air or glass. Note an insignificant fall in fibrinogen and a small mortality. Group C did not have any blood returned at the end of the shock period. Note that fibrinogen did not fall significantly and there was no mortality.

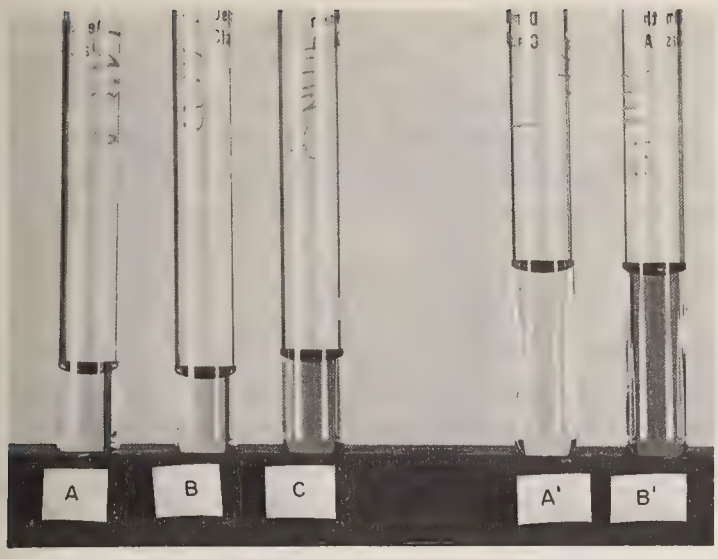


FIGURE 4.—On the left are three blood samples taken from a dog subjected to one hundred blows of a padded mallet on one thigh. The first tube was drawn before the trauma, the second tube 30 minutes following the trauma, and the third tube 48 hours after the trauma. Note that hemolysis, which was only mild right after the trauma, had increased markedly by the end of two days. This is thought to be due to absorption of hematoma and ecchymosis probably via lymphatics. If no hemolyzed blood had been absorbed, the mild hemolysis shown in the second tube would have completely disappeared in a few hours. On the right are two tubes drawn before and after the administration of 20 ml of autogenous blood which had been frozen and thawed. Note that the amount of hemolysis 48 hours after trauma and immediately after the administration of 20 ml of hemolyzed blood is approximately the same.

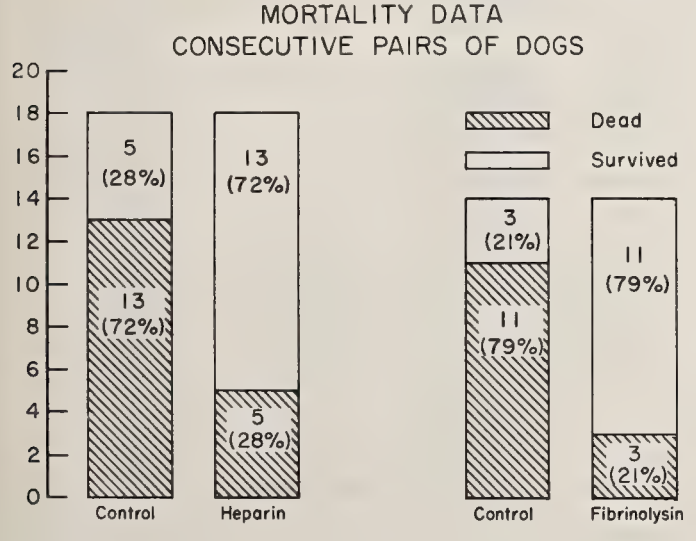


FIGURE 5.—Proctection by heparin and fibrinolysin of dogs subjected to "irreversible" hemorrhagic shock.

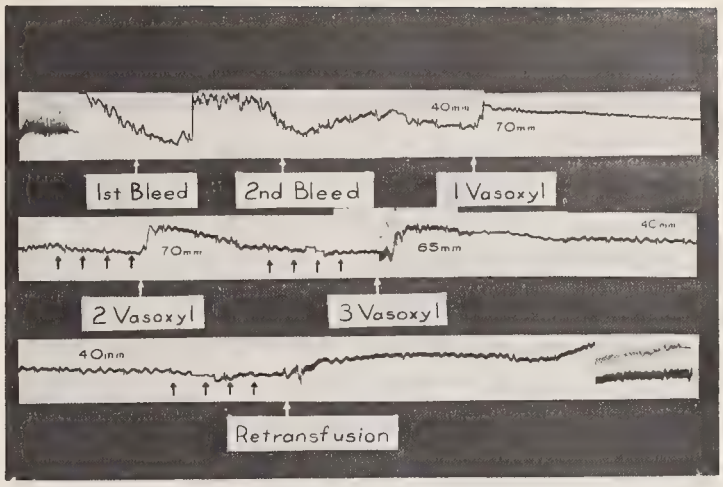


FIGURE 6.—Aortic blood pressure of dog subjected to hemorrhagic shock at 40 mmHg arterial pressure for four hours and treated with an agent which causes pure peripheral vasoconstriction without any cardiac effect. Upper panel shows results of initial and second bleed. Abrupt elevation between the first and second bleed is result of change in scale. Each small arrow represents the administration of 40 ml of blood. Note that administration of first dose of methoxamine (Vasoxyl) results in elevation of mean pressure from 40 to 70 mmHg. Although no blood was withdrawn to restore pressure to 40 mmHg, the pressure spontaneously fell to below 40 mmHg and required several transfusions of approximately 160 cc each to maintain pressure at 40 mmHg. Note that subsequent doses of the drug resulted in a temporary elevation of pressure but that pressure quickly fell even though no blood was withdrawn. These dogs died.

found after indotoxin administration. It was also found that heparin was inactivated in the presence of a pH below 7.2. In severe shock the capillary and tissue pH not only went below 7.2, but in severe shock reached levels 6.5 or below. These severe levels were not indicated by pH determinations of either arterial or venous blood, but required tissue pH determinations to document. This extreme acidosis was caused by the tremendous outpouring of lactic acid as the result of anaerobic cellular metabolism.

Working on the theory that DIC required two separate factors for its production, namely slow blood flow and a thromboplastic agent, it was thought that DIC could be prevented by promoting adequate flow even in the face of thromboplastic agents. With this in mind, it was decided if the arterioles could be opened up instead of being violently constricted due to

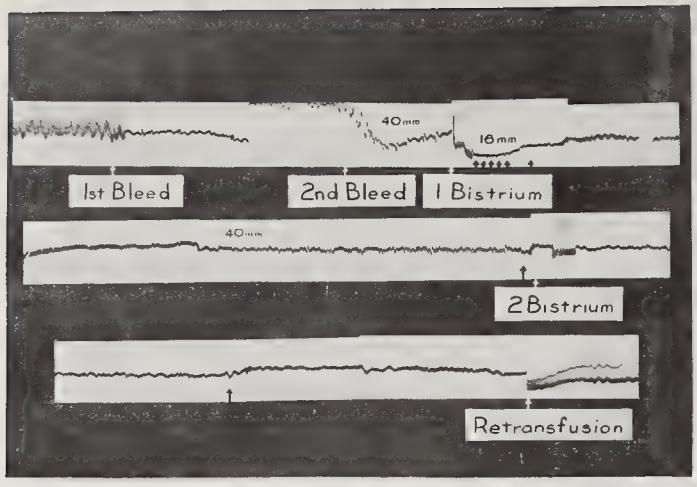


FIGURE 7.—Aortic blood pressure of dogs subjected to hemorrhagic shock at 40 mmHg arterial pressure for four hours and treated with a ganglionic blocking agent. Upper panel shows results of initial and second bleed. Abrupt elevation between the first and second bleed is result of change in scale. Each small arrow represents the administration of 40 ml of blood. Note that administration of first dose of hexamethonium (Bistrium) resulted in a dramatic fall in pressure which was restored to 40 mmHg by the administration of 160 ml of blood. Without this transfusion the animals would have died immediately. A pressure of 40 mmHg was maintained throughout the four-hour shock period with only 80 ml of additional blood. Note that the second dose of the drug resulted in no essential change in blood pressure. These dogs recovered.

high levels of catecholamines in shock, that the flow would be much improved in the capillaries. Consequently a series of experiments in dogs was set up to test this hypothesis. It was found that a vasodilating agent, if supported by even a minimal amount of blood volume (much less than given under controls), would not only prevent DIC, but would indeed prevent mortality (Figures 6, 7 and 8).[12] Not only did vasodilators prevent mortality, but they prevented the development of the coagulation defect which normally accompanies DIC (Figure 9). This was accomplished even though the vasodilator treated animals were actually given less blood and had less blood volume than did the control animals (Figure 10).

All of the above determinations have now been repeated and documented in human beings. The primary difference found between humans and dogs is the relative coagulability of the two species. For instance, the prothrombin time in dogs is roughly 6–7 seconds, whereas in humans it is approximately 13 seconds. Therefore, the same phenomena that takes place in dogs occurs in humans, but requires either more severe shock or more thromboplastic agent or both.

SUMMARY

Animals have been utilized as the primary source of scientific observations on shock until 1964. Valuable information was obtained, limited only by the known and unknown differences between animals and humans. In 1964, human studies in shock became practical for the first time, placing animal studies in a position of secondary importance. However, continued studies in animals will always be required to test new ideas, with primates probably playing an increasingly important role.

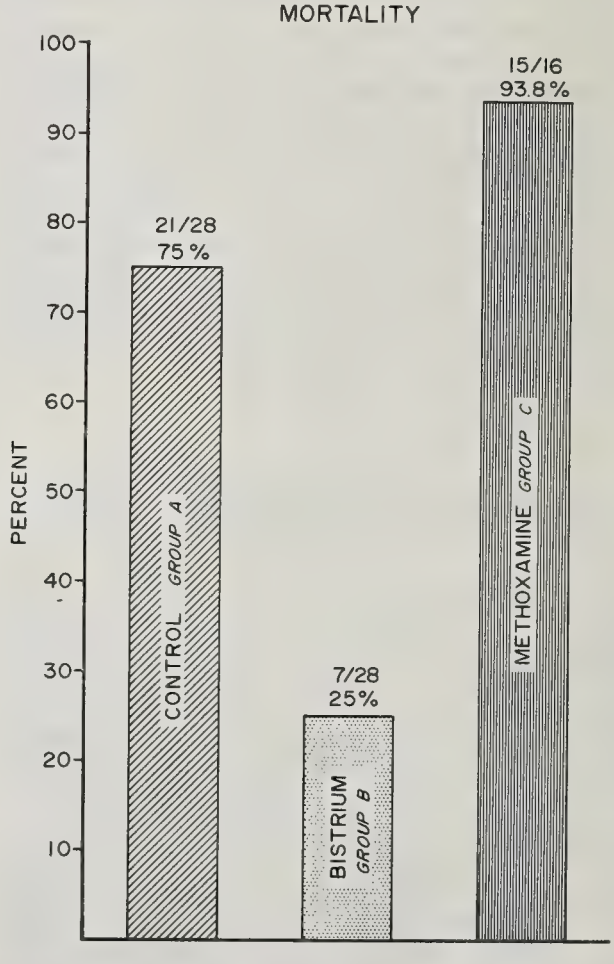


FIGURE 8.—Mortality rates of three groups of dogs subjected to hemorrhagic shock. All were maintained at 40 mmHg arterial pressure for four hours. Group B was treated with a ganglionic blocking agent and Group C with a peripheral vasoconstrictor.

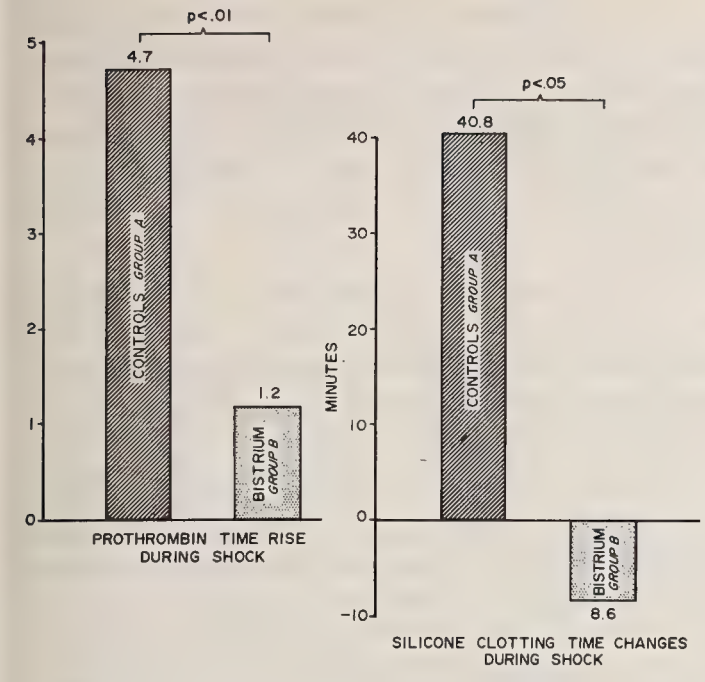


FIGURE 9.—Prothrombin time rise and silicone clotting time changes during hemorrhagic shock in dogs treated with vasoconstrictors or vasodilators. Note that the control group showed a marked lengthening of silicone clotting time as a result of the shock whereas group B dogs showed an actual slight shortening. That of Group C (vasoconstrictor) dogs approximated the time of the controls.

REFERENCES

1. CRILE, G. W. An Experimental Research into Surgical Shock. Philadelphia. J. B. Lippincott Co. 1899.
2. WIGGERS, C. J. Physiology of Shock. Cambridge, Mass. Harvard University Press, 1950.
3. HEWSON, W. in The Works of William Hewson. FRS G. Gulliver Ed. London Sydenham Society 1946, p 46.
4. HARDAWAY, R. M. et al. Studies on the Role of Intravascular Coagulation in Irreversible Hemorrhagic Shock. Ann. Surg: 155, p. 241–250, 1962.
5. WIGGERS, C. J. Physiology of Shock. Cambridge, Mass. Harvard University Press, 1950.
6. HARDAWAY, R. M. et al. The Influence of Extracorporeal Handling of Blood on Hemorrhagic Shock in Dogs. Exper. Med & Surg, 23, 28–37, 1965.
7. HARDAWAY, R. M. Syndromes of Disseminated Intravascular Coagulation. Charles Thomas, Springfield, Ill. 1966.
8. HARDAWAY, R. M. et al. Influence of Trauma and Hemolysis on Hemorrhagic Shock in Dogs. J. of Trauma, 4(5), 624–641, 1964.
9. HARDAWAY, R. M. and BURNS, J. W. Mechanism of Action of Fibrinolysin in the Prevention of Irreversible Hemorrhagic Shock. Ann. Surg. 157: 305–309, 1963.
10. HARDAWAY, R. M. and JOHNSON, D. Clotting Mechanism in Endotoxin Shock. Arch. of Int. Med. 112: 775–782, 1963.
11. HARDAWAY, R. M. et al. Clotting time of Heparinized Blood, Influence of Acidosis. Arch. Surg 89: 701–705, 1964.
12. HARDAWAY, R. M. et al. Influence of Vasoconstrictor and Vasodilators on Disseminated Intravascular Coagulation in Irreversible Hemorrhagic Shock. Surg. Gyn and OB. 119: 1053–1961, 1964.

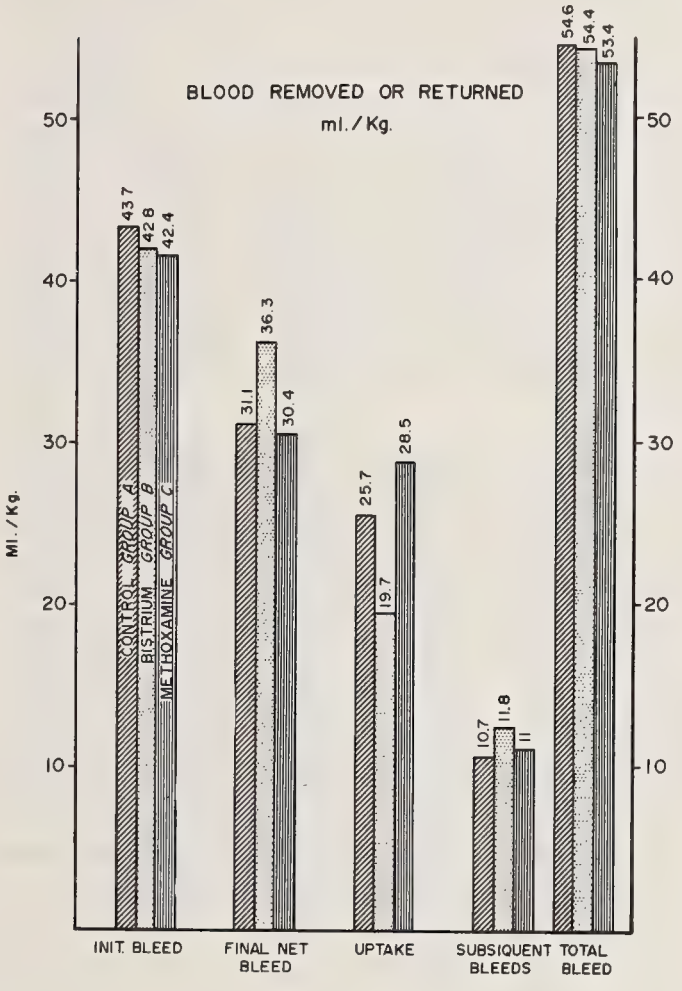


FIGURE 10.—Summary of blood removed or returned during hemorrhagic shock in dogs treated with a vasodilator or a vasoconstrictor. The initial bleed was that required to lower the arterial blood pressure to 40 mmHg. Final net bleed was the amount of blood remaining outside the animal at the end of the shock period. Uptake is that amount of blood required to be administered during the shock period to maintain pressure at 40 mmHg mean pressure. Subsequent bleed is the amount of blood withdrawn after the initial bleed to keep blood pressure down to 40 mmHg. Usually this was withdrawn during the early part of the shock period and uptake took place during the latter part of the shock period. Total bleed is the sum of the initial and subsequent bleeds.

DISCUSSION

CHAIRMAN KEZDI: Dr. Hardaway, do you think that in hemorrhagic shock, extreme vasoconstriction, capillary stasis and sludging contribute to some problems which relate to the deterioration of these animals?

R. M. HARDAWAY: Yes, unquestionably. In fact, shock essentially is a vasoconstriction with an accompanying and consequent slowing of capillary blood flow. The vasoconstriction pinches off some of the flow in the body's attempt to maintain adequate blood pressure. It is a good first aid mechanism. It keeps the blood pressure near-normal, but it is not satisfactory as a long-term measure. In other words: if the vasoconstriction persists for any length of time, it becomes a negative instead of a positive value and the first organs to deteriorate are those which are most vasoconstricted, namely the kidneys and some of the other vital organs.

I therefore believe that vasoconstriction continued over a period of time is very definitely a detrimental factor and should not be allowed to continue. It is usually best prevented by just giving volume; this will cause a fall of the catacolamine level. We have, in fact, abundant data on this: when the catacolamine level is high, it returns to normal as fluid is given. A physiologic vasodilation occurs simultaneously with the above.

CHAIRMAN: That is why vasodilators and volume extension work.

C. G. LA FARGE, Children's Hospital Medical Center, Boston, Mass.: You mentioned pretreatment with good results when using fibrinolysin in animals. Did you use fibrinolysin, urocarnase or something of that type ex post facto while you had the already developed deteriorated state?

R. M. HARDAWAY: I used fibrinolysin preterminally in a few clinical cases. I have usually been afraid to give fibrinolysin clinically because these patients always have a completely incoagulable blood at that time.

If one gives even a mild or small dose of fibrinolysin in the presence of a hypercoagulable state and in the presence of shock, it will reduce the fibrinogen to zero. I have been afraid to try this, even though theoretically, it would dissolve the clots. Heparin is somewhat similar; it is even worse, however, because it does not dissolve already formed clots. Nor does it do anything to prevent platelet agglutination, which I believe is perhaps the first step in coagulation. Thirdly, it is inactivated in any pH levels which we have to cope with in the capillaries. Heparin, I believe, is currently given to prevent DIC, particularly in shock due to meningococcal septicemia, apparently with clinically good results. But I have been personally afraid to use it. I think that it is much easier and safer and perhaps more effective to promote blood flow by means of adequate volume and, if necessary, vasodilators, since they will prevent DIC.

OBSERVATIONS ON EXPERIMENTAL MYOCARDIAL INFARCTIONS IN DOGS

Jacob I. Hirsch, Fritz Streuli and Fred Gorstein*

During development of a standardized reproducible method for producing coronary thrombosis and myocardial infarction in closed chest, anesthetized dogs, a number of accompaniments of the pathologic sequence were studied.

Electrocardiographic pattern changes which accompanied myocardial infarction in the dog were comparable to those in man. In almost all experiments early ventricular arrhythmias appeared about one-half hour after infarction, and lasted about 2 hours. Although easy to control with intravenous lidocaine, these were potentially lethal. After about an hour or two of quiescence, late ventricular arrhythmias appeared, lasting as long as 72 hours. These were quite resistant to therapy with lidocaine, but have proven to be fairly innocuous.

Among the serum enzyme changes, CPK elevation appeared first accompanying infarction. LDH elevation was last to appear. The ratio of peak levels to pre-infarction levels is greatest for CPK and least for LDH.

Hemodynamic measurements revealed impairment of the circulatory state following acute infarction, slightly more than in sham operated dogs. The tendency to return toward normal was less for infarcted dogs than for sham operated ones. LVEDP showed the greatest change, and remained abnormal during the healing period.

At autopsy, infarcts were located in regions predictable from a knowledge of the site of occlusion, but their sizes were much less predictable either from coronary arteriographic information or from electrocardiographic pattern changes. The elevations in serum enzyme activities, especially of CPK, were good predictors of infarct size. With one exception, myocardial infarction and CPK elevation were either present or absent together.

INTRODUCTION

Numerous methods have been used to occlude coronary arteries and to produce myocardial infarction in animals. We have chosen the method first described by Salazar.[1] This method consists in passing a low intensity direct current between 2 electrodes, one placed on the skin and a second in a major branch of a coronary artery. This method was deemed to be uniquely attractive because of a number of features which it embodies. The first is obviating thoracotomy and pericardiotomy, with their attendant influences upon hemodynamic, metabolic and electrocardiographic changes, changes in acute phase reactants and a possible effect upon collateral vasculature.[2] The second is the assurance that *in vivo* thrombosis at the site of intimal arterial damage is the means by which the artery is occluded. The third is the gradual occlusion of the artery which occurs, and which is controllable to some extent.

This method of producing coronary occlusion by thrombosis and myocardial infarction appeared highly attractive in comparison to other methods. Early reports, however, contain no definite information concerning the reproducibility of the coronary lesion, myocardial infarction, and the changes or predictability of the electrophysiologic, hemodynamic and metabolic responses. In these experiments, we attempted first, to modify the experimental intervention in ways which would reveal the optimal conditions for producing arterial damage, thrombosis, and myocardial infarction, and, second, to evaluate the results of this experimental intervention. The changes produced could then be evaluated with regard to their reproducibility, predictability, quantitation and correlation with the experimental intervention. Occlusions were attempted a 91 sites in the coronary arteries of 80 dogs. In 5 of these dogs occlusion was attempted at 2 sites and in 3 of these dogs occlusion was attempted at 3 sites.

METHODS

Eighty mongrel dogs were used, of which 33 were females, and 47 males. Their weights ranged between 26 and 70 pounds. The dogs were lightly anesthetized, using pentobarbital anesthesia, or pentobarbital plus morphine sul-

* Department of Medicine, New York University School of Medicine

fate, 0.25 mgm per pound. The dogs were artificially ventilated with room air by means of a Harvard animal respirator pump. The experiments were divided in general into 2 categories: acute, in which the planned survival was 4 hours or less, and chronic, so-called, in which planned survival was 1 to 7 days. There were 49 acute experiments and 27 chronic experiments attempted, totaling 76 dogs. The 4 remaining animals were excluded from further consideration because in 2 cases the coronary artery system could not be entered with a catheter, and in the remaining 2 cases technical problems necessitated termination of the experiments.

General Techniques

Certain general experimental methods, common to all experiments, were followed in the course of preparing and carrying out these studies. In the acute experiments, catheters were passed into the great cardiac vein and into the coronary artery via the superficial jugular vein and common carotid artery, respectively. A third catheter was passed into the descending thoracic or abdominal aorta via the right femoral artery. The catheter in the coronary artery was used to pass the electric current and cause thombosis. The catheters in the cardiac vein and aorta were used to obtain A-V differences across the myocardium, to monitor arterial blood pressure and to administer drugs as required. In the chronic experiments, in which complete hemodynamic studies were performed, the locations of the catheter tips and their sites of entry were the coronary sinus and great cardiac vein via the left jugular vein, the pulmonary artery via the right jugular vein, the left ventricle via the left carotid artery, and later, replacement of the left ventricular catheter by the catheter into the left anterior descending coronary arery via the left carotid artery. A short catheter was also passed to the root of the aorta via the right carotid artery for determination of ejection factions. All catheters were kept patent by slow infusion of normal saline. Heparin in a concentration of 20 mgms per liter was added to the saline which was used to infuse the cornary sinus catheter, since clotting was found to be a problem in this site. The first day in the chronic experimental animals required between 12 and 14 hours, during which time continuous anesthesia was maintained. A total of between 2 and 2½ liters of saline was infused during this time. Blood loss due to sampling totaled between 100 and 150 milliliters during the same period of time, and this blood loss was not replaced.

All arteries and veins were ligated at the site of entrance of the catheter introduced into them, except for the femoral artery which was repaired after the catheter was removed. None of these ligatures were removed, including the ligatures around both common carotid arteries in the chronic experiments. Even with bilateral ligation of carotid arteries and jugular veins no neurological deficit was apparent up to the time of sacrifice in any dogs.

Antiarrhythmic therapy with lidocaine was administered only as required to control or abolish arrhythmias after they appeared. Doses of 25–75 mgms of this drug were injected acutely intravenously when arrhythmias appeared, especially in the first hour following myocardial infarction. Since the surgical techniques involved clean but not sterile methods, dogs which were to survive in the chronic category were given 1 gm of ampicillin or penicillin G intravenously near the conclusion of the first day of experiment. This proved to be effective in preventing wound infections.

Rectal temperature was monitored with a continuous indwelling thermocouple, and blankets were applied as necessary when deep body temperature tended to fall.

Regional blood flow through the branch of the coronary artery system in which the catheter was located was estimated semiquantitatively by fluoroscopic observation of the rapidity with which Hypaque was washed out of the artery following its injection.

Traumatic Interventions

With regard to the traumatic interventions necessary to cause coronary thrombosis and myocardial infarction, the following 4 variables were studied in an attempt to gain insight into the optimal conditions necessary for causing this pathological sequence of events.

1. The effect of anodal versus cathodal current.

2. The effect of pairing the intracoronary electrode against a single precordial electrode versus 6 external electrodes arranged around the circumference of the chest.

3. Varying the total energy delivered.

4. Observation on the strenth and duration of the electric current using weak currents of less than 1 milliampere for 5 minutes or more versus intense currents delivered in pulses of 11 milliseconds each.

Pathophysiologic Consequences

The effect of thrombotic occlusion of a branch of the coronary artery system upon 5 variables are cited.

The first is the electrocardiographic, in which changes in pattern and in rhythm were monitored. In 5 of the so-called chronic experiments, the electrocardiogram was recorded continuously on magnetic tape for 24 hours following delivery of electric current into the coronary artery.

The second group is the serum enzymes including creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT), and lactic dehydrogenase (LDH), which were measured at intervals in 37 dogs. In 25 of these, A-V differences were measured between arterial blood and coronary sinus blood.

The third is A-V differences in serum lactate concentration between arterial and coronary sinus blood, which were determined in 7 experiments. In 5 of these myocardial infarction occurred, and in 2 it did not occur. In all of the lactate studies, serum samples were drawn 70 minutes and 15 minutes prior to initiation of electric current, and 60, 90, 120 and 150 minutes following the beginning of electric current.

The fourth group of sequelae of coronary occlusion and myocardial infarction comprises changes in hemodynamics. Heart rate (HR), cardiac output (CO), ejection fraction (EF), maximum isovolumic pressure (MIP), maximum rate of pressure rise (dp/dt), aortic pressure (AP) and left ventricular pressure (LVP) were measured. In addition, stroke volume (SV), end-diastolic volume (EDV), left ventricular circumference (LVC) and contractility index (ConI) were calculated.[3] Cardiac output, stroke volume, end-diastolic volume and ejection fraction were determined by means of the dye dilution method using a Lexington Analog Computer and Cardiogreen dye.

The fifth group of observations made following myocardial infarction consists of the pathologic changes which occurred in the coronary artery and in the myocardium. Following sacrifice, hearts were fixed in 10 percent formalin. Fifty-nine of these were prepared for histological examination in addition to gross examination. The coronary arteries and veins were examined in all cases. The myocardium was examined in all cases except those in which death occurred very early in the experiment. Examination of the coronary arteries and veins included tissue blocks proximal to, at, and distal to the site of occlusion. In the chronic experiments, the fixed hearts were sliced in parallel coronal sections 1 cm thick. The surfaces of these sections were photographed and then traced, and the infarction mapped using gross and histologic findings.

RESULTS

General Techniques

Depending upon the degree to which the Sones catheter was wedged within the coronary artery, the rate of clearance of injected Hypaque was either very rapid, slowed to varying degrees or completely arrested. We have found that for intimal damage and thrombus formation to occur, the rate of Hypaque clearance must have been reduced to a significant degree. In each experiment, therefore, this was achieved. When a substantial reduction in blood flow occurred, the electrocardiogram always manifested repolarization abnormalities due to the resulting myocardial ischemia.[4] If the flow was completely arrested a current of injury was observed after about 3 minutes. These changes were distinct from the transient, direct effect upon repolarization caused by the Hypaque itself.

Traumatic Interventions

A comparison of changing the polarity of the intracoronary electrode from anode to cathode demonstrated that both were effective in producing arterial lesions and thromboses. The slightly greater effectiveness of anodal current

was offset by the fact that cathodal current did not cause the electrolytic deposition of heavy metal salts from the wire within the artery. In 5 experiments in which 6 external electrodes were arranged circumferentially around the thorax, only 2 occluding thrombi were produced after delivery of an average electrical energy of 675 mw sec. Sixteen experiments in which the intracoronary electrode was paired with a single external electrode located on the chest overlying the anterior surface of the heart, culminated in 13 occluding thrombi after an average energy delivery of 317 mw sec. The latter technique was therefore selected for all subsequent experiments.

The total energy delivered between the intracoronary electrode and the external electrode to a tissue load of approximately 500 ohms, ranged from 18 to 2067 mw sec. We have found that the production of arterial wall damage and thrombosis depended in each individual experiment upon attaining the prerequisite amount of blood flow reduction. In the experiment cited above where 2067 mw sec. of total energy was delivered, blood flow could not be reduced sufficiently to allow either arterial wall damage or thrombosis to occur. In contrast, where a good reduction in blood flow was attained, as little as 54 mw sec. of total electrical energy produced intimal damage and complete occlusion by thrombus. In any given dog, continuation of electric current caused increasingly persistent manifestations of ischemia on the electrocardiogram. Therefore, in each experiment the termination of electric current delivery depended upon electrocardiographic changes which were sustained for 20 minutes or more without further electric current. The average amount of energy required for thrombotic occlusion was about 400 mw sec, and the magnitude of the curents ranged from 0.2 to 1.0 milliamperes, and averaged 0.8 milliamperes delivered for an average of 20 minutes.

In contrast to the dimensions of electric current described above, 9 experiments were performed utilizing a specially constructed current generator which delivered impulses 11 msec in duration and up to 320 ma strength. The total energy delivered ranged from 1 to 5120 mw sec, and in 1 experiment 60,000 mw sec were delivered. Despite the fact that the current pulses were synchronized to occur during the refractory period of the ventricular myocardium, 2 dogs died with ventricular fibrillation immediately following delivery of current. A third dog died of ventricular fibrillation 15 minutes after electric current delivery. In the experiment in which the energy dose was 60,000 mw sec, an intimal lesion and a partially occluding thrombus were found at autopsy. In only one other heart was a minor intimal lesion found accompanied by a totally occluding thrombus, and in a third heart a partially occluding thrombus was found but no evidence of intimal damage. The remaining 6 hearts showed neither intimal damage nor thrombus formation. In addition to the obvious conclusion that this method of arterial injury was inferior to the former one, the fact that the electric energy was delivered over a much shorter period of time mitigated against the possibility that the mechanism of arterial injury was a thermal one. Since the average administered electrical energy of 400 mw sec cited above, has a heat equivalent of 0.1 calorie, it was also felt on a theoretical basis that the mechanism of arterial injury was not a thermal one.

Pathophysiologic Consequences

The electrocardiographic manifestations of acute myocardial infarction followed the same sequence as the classical changes observed in man. Very transient peaking of T waves was followed by S-T segment elevations of varying magnitudes. All S-T segment displacements returned to the baseline. Progressive loss of R wave amplitude, increasingly deep and wide Q waves and terminal inversion of T waves proceeded simultaneously. The distribution of these changes led to interpretations ranging from anteroseptal to anterolateral locations of the acute infarct, in agreement with the fact that the occlusion were located in the mid left anterior descending coronary artery or one of its major branches, in all of our experiments.

We found surprising uniformity between experiments in the electrocardiographic manifestations of infarction, in contrast to the wide and varied deviations from the so-called "textbook picture" of myocardial infarction seen in

human clinical cases. We also observed a markedly accelerated course of evolution of the pattern of infarction compared to clinical human cases. Q waves of diagnostic dimensions were typically well established in 3 to 4 hours following myocardial infarction, and by 8 to 12 hours all remnants of injury currents had usually disappeared. In none of our experiments did myocardial infarction produce supraventricular arrhythmias, atrio-ventricular block or intraventicular block.

A number of interesting and somewhat puzzling observations should be mentioned. In 6 experiments S-T segments were observed to remain elevated for 3 hours until the time of sacrifice, and no myocardial infarction was found at a time when early changes of infarction should have been evident. In 5 of these 6 cases serum enzyme levels did not exceed normal limits, lending further support to the anatomic observation. Continued S-T segment elevation for 3 hours is therefore not necessarily an indication that acute infarction has occurred. In 1 experiment, only, a large transmural infarct was unaccompanied by any changes in ventricular depolarization. On the other hand, 2 cases in which scattered focal myocardial necrosis was found were accompanied by pathological Q waves. In the most interesting and inexplicable case, diagnostic Q waves appeared despite the absence of infarction pathologically and the absence of rise above normal of the CPK.

Ventricular arrhythmias ranged from premature contractions to ventricular fibrillation. These arrhythmias were clearly separable into two groups, early and late. The early arrhythmias characteristically made their appearance 15 to 30 minutes after the appearance of injury currents and subsided in 1½ to 2 hours. These were found to be potentially lethal, but typically were easily suppressed by prompt administration of intravenous lidocaine. The group of late ventricular arrhythmias typically made their appearance about 2½ to 3 hours following coronary occlusion after an intervening quiescent period of a ½ to 1½ hours. These arrhythmias persisted for 1 to 3 days, were extremely resistant to control with intravenous lidocaine but with 2 exceptions were non-lethal and well tolerated.[5-7]

In our initial experiments, the levels of 3 enzymes, creatine phosphokinase, glutamic oxoloacetic transaminase, and lactic dehydrogenase were measured in the arterial blood and in the myocardial venous drainage. The latter samples were obtained from catheters in the proximal great cardiac vein to favor insofar as possible venous drainage from the region of infarction. Table I demonstrates the average levels of enzyme activity of 6 dogs that sustained large myocardial infarctions. The time of onset of acute myocardial infarction in this table and in all our experiments was defined as that time in which the electrocardiogram first showed a current of injury which persisted. CPK activity in the cardiac venous blood showed a small but definite rise 1 hour following zero time, thus defined. From 2 hours onward the arterial blood levels were elevated well into the abnormal range. Maximum A-V difference across the myocardium occurred at 3 hours. In contrast, the GOT level in cardiac venous blood had risen significantly by 2 hours post-infarction. Arterial blood levels were also minimally elevated by

TABLE I.—*Enzymes (mU/ml) (Average of 6 chronic dogs with large myocardial infarction)*

TIME TO MI	CPK			GOT			LDH		
	ART.	CS	CS-A	ART.	CS	CS-A	ART.	CS	CS-A
−3 hrs.	67	60	− 7	10.1	12.9	+ 2.8	197	198	+ 1
−1½ "	56	52	− 4	13.4	12.7	− 0.7	150	190	+ 40
−½ "	55	53	− 2	13.0	15.7	+ 2.7	154	168	+ 14
0				ACUTE MYOCARDIAL INFARCTION					
+1 "	74	98	+ 24	19.0	20.5	+ 1.5	166	192	+ 26
+2 "	323	514	+191	46.0	61.0	+15.0	276	290	+ 4
+3 "	1221	1678	+457	114	140.0	+26.0	471	573	+102
+4½ "	2166	2320	+154	180	200.0	+20.0	764	868	+104
+6 "	2578	2823	+245	247	254.0	+ 7.0	788	930	+142
+7½ "	2814*	—	—	253 *	—	—	831*	—	—
+9 "	3326*	—	—	264 *	—	—	841*	—	—
+24 "	1750*	—	—	146 *	—	—	382*	—	—

* Mixed venous (instead of arterial) samples

this time. The peak A-V difference for GOT occurred 3 hours post-infarction. LDH activity in both the arterial blood and the cardiac venous blood exceeded upper normal limits at 2 hours post-infarction, but a definite A-V difference was not seen until 3 hours. The peak A-V difference occurred at 6 hours, the last time for which venous samples were obtained. The ratio of observed peak values to baseline values of enzyme activity for CPK, GOT and LDH were 55, 22 and 5, respectively.

All 33 experiments in which CPK activity in mixed venous or arterial blood rose to abnormal levels, showed myocardial infarcts at autopsy. Five experiments, during which CPK levels did not exceed upper normal limits and in which myocardial infarction was attempted, failed to show any evidence of infarction at autopsy. In 1 experiment in which CPK activity rose to abnormal levels, no myocardial infarction was found. The possibility that an infarction was missed cannot be excluded. With this one exception it appeared that myocardial infarction and CPK elevation were either both present or both absent.

Figure 1 correlates serum CPK levels with EKG changes. It was found that changes in repolarization in the electrocardiograms preceded elevations in CPK activity, but that elevations in CPK activity preceded abnormalities in depolarization. By the time depolarization changes were manifest, CPK levels had exceeded 500 mU/ml.

Lactate levels were determined in the arterial and coronary sinus blood of 7 dogs, 5 with acute myocardial infarction and 2 without. The control dogs showed no appreciable change in lactate levels in either arterial or coronary sinus blood throughout the course of the experiment. The 5 dogs with myocardial infarction were indistinguishable from the control group in that none showed any change in coronary sinus lactate during the post-infarction period, compared to the pre-infarction period. It is emphasized that the venous samples were mixed myocardial venous blood. However, as in the case of studies of enzyme levels, the coronary sinus catheter was advanced far into the proximal great cardiac vein to enhance selective venous drainage from the anterior aspect of the left ventricle, where the infarcts were produced.

The results of hemodynamic measurements in 6 infarcted dogs and in 2 sham operated dogs are summarized in Figure 2. The CO, SV, EF, dp/dt, EDV and ConI all show a fall with respect to control values 2½ hours post-infarction, and at a comparable time in the sham operated dogs. At the same time both the infarcted group and the sham operated group demonstrated increases in HR and increases in LVEDP. The infarcted group, in general, showed these changes to a greater degree. The most distinct difference between the 2 groups, however, is seen in the data obtained 3 days following myocardial infarction or sham operation. In the former group there was a return toward normal but the preinfarction values were never achieved. In the sham group, these variables returned closer to or even beyond control values. The single variable which became most abnormal 2½ hours following infarction and which showed the least tendency to return to normal 3 days later was LVEDP.

It can be seen that not only in the infarcted dogs but in the sham operated dogs, general deterioration of hemodynamic state was apparent at the 2½ hour point. Anesthesia, surgical manipulations, withdrawal of blood samples and injection of Hypaque into the coronary arterial system were the major factors which the sham group shared with the infarcted group, and which were found to influence the hemodynamic state. Of interest is the observation that in this group of infarcted dogs where 15 to 25 percent of left ventricular mass was destroyed, the hemodynamic deterioration at 2½ hours or at 3 days was not more profound.

Our pathologic findings consisted of two categories—the myocardial, in which examination was oriented to mapping the size and location of the myocardial infarction, and the coronary arteries, in which presence and severity of vascular damage and presence and extent of intravascular thrombosis were studied. The upper limit of the locations of infarcts was the anterior aspect of the left ventricle approximately midway between the coronary ostium and the apex of the heart. The lower limit was the apex proper, occasionally with involvement of the diaphragmatic surface of the apical region. The

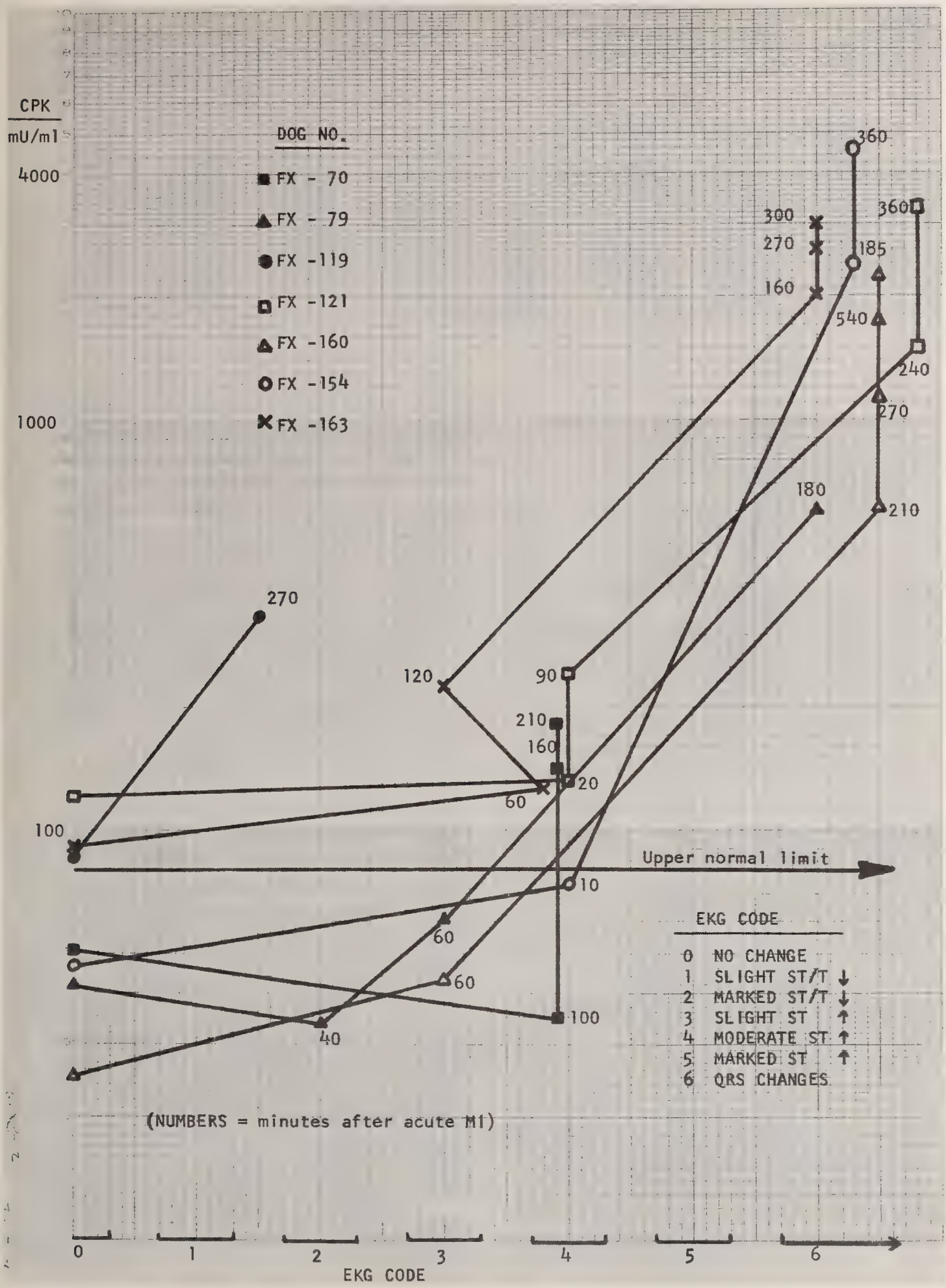


FIGURE 1.—Correlation of serum CPK levels with EKG changes.

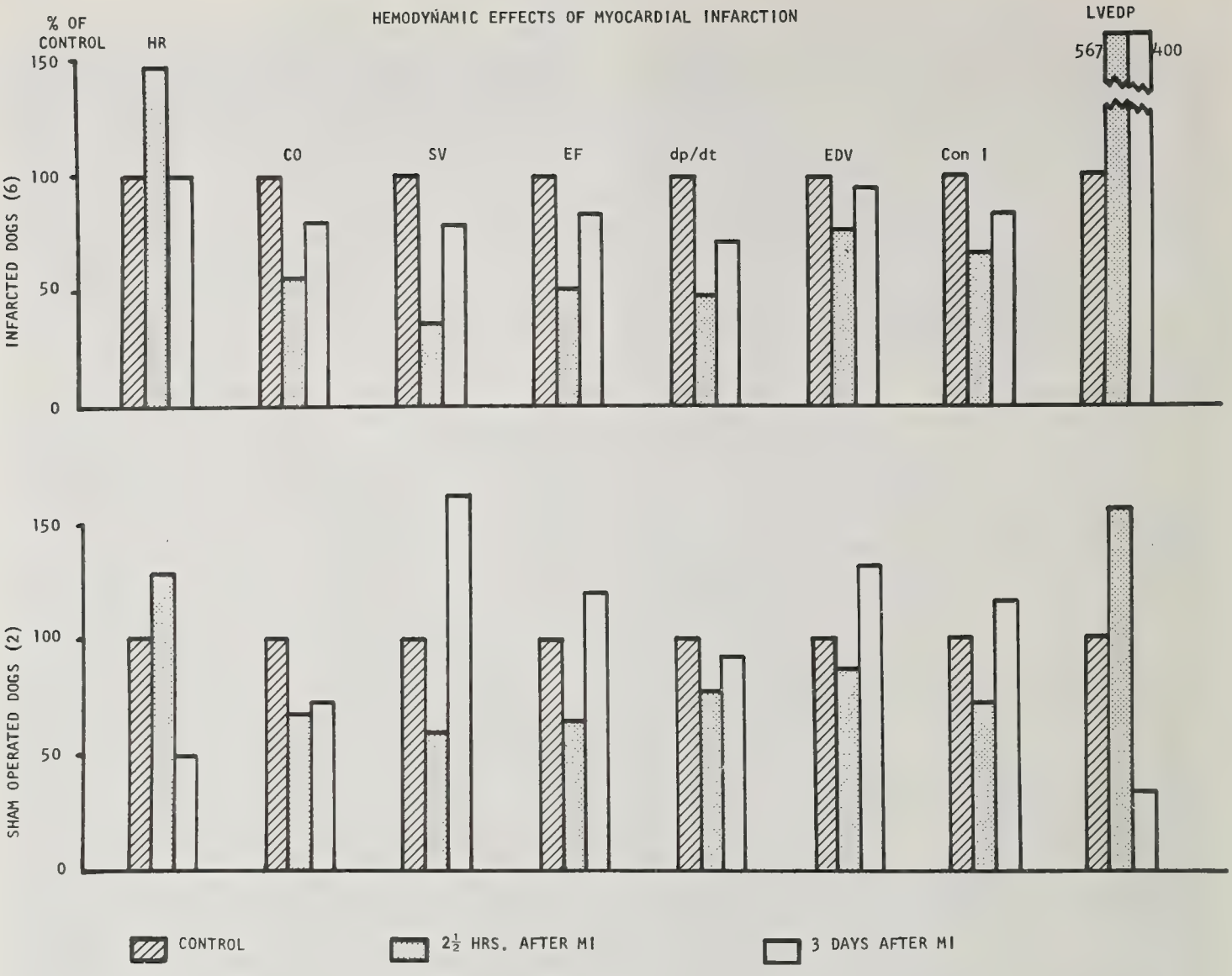


FIGURE 2.

medial limit included the interventricular septum midway between its junction to anterior and posterior walls of the heart. The lateral limit was the anterior 50 percent of the free wall of the left ventricle. Expressed as a percentage of left ventricular mass, infarct sizes ranged between 5 and 35 percent.

The expected observation was made that the location of the infarction paralleled closely the site of occlusion in the coronary artery system with the more laterally located infarcts related to occlusion of diagonal branches and the more anterior and anteroseptal locations more related to occlusion of the main LAD coronary artery. Actual size of infarction, however, was much less related to the size of the vessel occluded than would have been anticipated. Occlusion of vessels of comparable sizes were found to produce infarcts of quite widely variable size, ranging from small, focal, subendocardial lesions, to massive transmural ones.

The lesions which have been observed in the coronary arteries range in size and degree from minor intimal changes to large zones of frank wall destruction. Contrary to the original observations of Salazar, we have found a poor relationship between the extent of vessel wall damage and the total amount of energy delivered. Comparison of anodal and cathodal current also revealed little difference. Arterial wall lesions were always localized, and limited typically to the pericardial aspect of the arterial circumference. This correlated with the region of highest current density. With two exceptions

intra-arterial thrombi were always associated with arterial wall lesions. It was clear that a wall lesion was a necessary prerequisite for thrombus formation. All of the thrombi produced by this method appeared microscopically to be of the classical structure associated with an *in vivo* thrombus, including platelets, fibrin and other cellular components, and were clearly distinct from a simple clot. Where partially occluding thrombi were found, the site of attachment to the vessel wall was always at the site of injury. We found that in the course of 7 days, the structure of the intra-arterial thrombus was not static, but that initially it was composed mainly of densely packed platelet aggregates surrounded by strands of fibrin. Later platelets disintegrated and more fibrin was visible within the thrombus, and finally leukocytes appeared on and within the thrombus mass. After 2 to 4 days, the amount of fibrin was reduced as early reparative processes became apparent. These observations were in agreement with those of Jørgensen et al. made in the carotid artery of the pig.[8] The sites of arterial damage and thrombus formation were typically just beyond artery bifurcations, as shown in Figure 3. In addition to the traumatic effect of electric current, such locations also satisfied the other prerequisite condition alluded to previously, namely, that significant reduction of blood flow occurred at these sites.

Figure 4 is a section of a 3-day old lesion stained with H and E. The arterial wall lesion is seen at the pericardial aspect of the vessel. A complete loss of smooth muscle structure is seen, producing a homogeneous, eosinophilic appearance. There was destruction of the internal elastic membrane with bleeding into the medial layers. Infiltration with leukocytes was apparent in this area.

Figure 5 shows a 4-day old lesion seen under low power. The thrombus which was present only partially occluded the lumen and originated from a localized lesion of the vessel wall. Figure 6 shows a 3-hour old process, with metalic salt visible within the thrombus mass, a finding typical of thrombi produced with the intra-arterial electrode of anodal polarity. Figure 7 shows a thrombus only 30 minutes old. Under low power it appears to be completely occluding the vessel lumen. The pericardial aspect of the artery appears to be the site of damage in this section.

DISCUSSION AND CONCLUSIONS

We have found in these experiments that the simultaneous occurrence of two factors was necessary for thrombus formation. These were: (1) a substantial reduction in blood flow, and (2) a lesion of the arterial wall. Based upon this, it is difficult to conceive how thrombosis could occur naturally in the presence of only

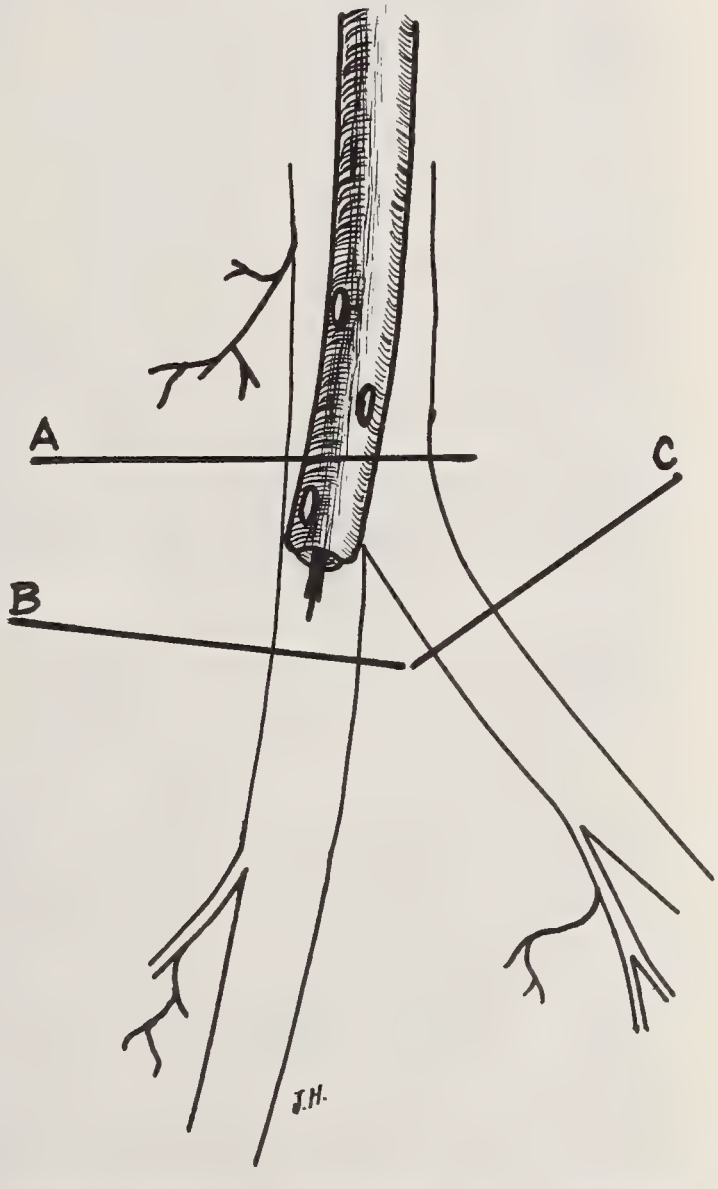


FIGURE 3.—Schematic representation of a wire-containing catheter wedged at point of bifurcation of a coronary artery. Cross sectional area at A equals sum of cross sectional areas at B and C. Ratio of diameters at A to B or A to C are 1.4 to 1 if B and C are of equal size. Flow through C is unmodified compared to flow through B.



FIGURE 4.—Section of 3-day old lesion. Arrow indicates area of bleeding and cellular infiltration into medial layer of artery. Epicardial aspect of artery is seen in upper left.

one or neither of these factors. The mechanism by which electric current caused the arterial lesion remains unexplained, although thermal effects were excluded to our satisfaction. The dependence upon degree of blood flow reduction thwarted our ability to prescribe a specific amount of total electrical energy necessary to cause thrombosis.

The sequence of the evolution of the electrocardiographic pattern of myocardial infarction followed the classical changes, but at a much accelerated rate, and was stabilized in the so-called chronic pattern within 12 hours. Two kinds of arrhythmias, always ventricular, were found. The early group subsided within 2 hours, were potentially lethal, but were easily controlled with intravenous lidocaine. The group of late ventricular arrhythmias appeared after about 1 hours of quiescence and lasted for as long as 3 days, were typically innocuous, and were also extremely resistant to control with intravenous lidocaine. The segregation of arrhythmias into early and late with a definite quiescent period between them was observed very frequently, and remained unexplained. It was not related to the size of the myocardial infarction.

Comparison of 3 cellular enzymes, released into the circulation, LDH, GOT and CPK, revealed the CPK to be the most sensitive accompaniment of infarction from the standpoint of the ratio of peak levels to baseline levels. Furthermore, whenever myocardial infarction was found, serum CPK activity rose to abnormal levels. Reversible myocardial injury, as judged

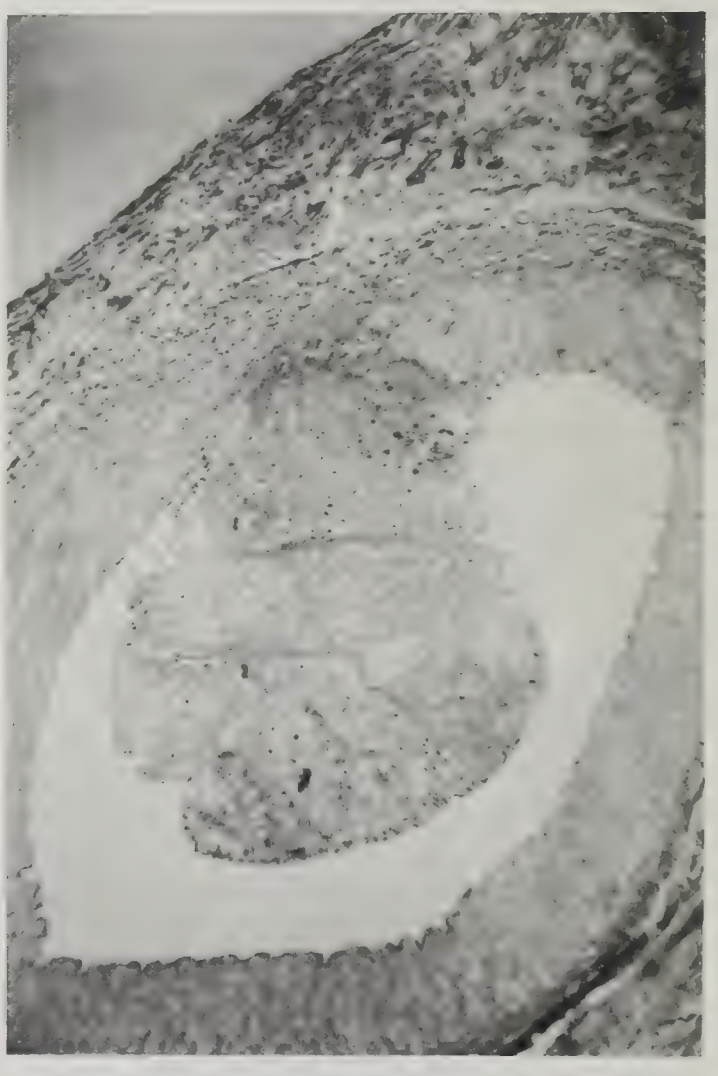


FIGURE 5.—Section of 4-day old lesion. Epicardial aspect of artery is seen in upper left.

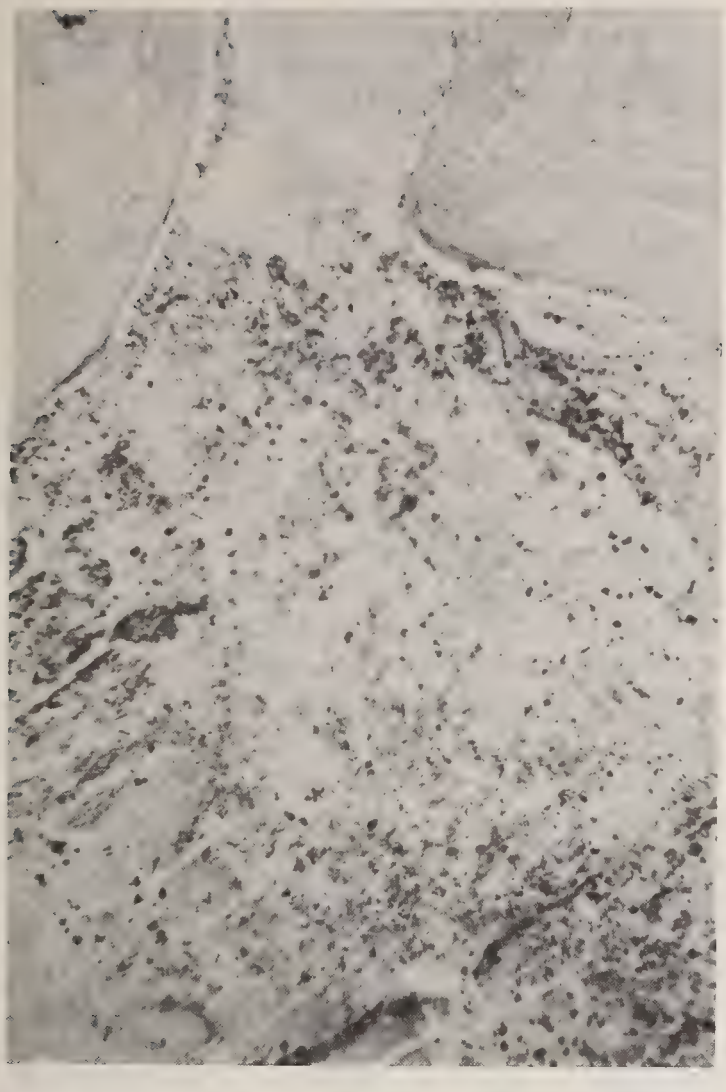


FIGURE 6.—Metallic salts appear as irregular black granules throughout thrombus mass.

by the electrocardiogram, was not accompanied by CPK release. CPK release was deemed to be a clinical *sine qua non* for myocardial necrosis. Shell et al. have compared quantitatively the size of myocardial infarction in the dog with the total CPK released, and these were found to agree closely.[9]

SUMMARY

In summary, our hemodynamic measurements in anesthetized dogs revealed a general depression of cardiac function immediately following myocardial infarction. This did not return completely to the pre-infarction status after 3 days, in contrast to sham operated animals. The most markedly disturbed of these variables was the LVEDP, which, after 3 days, remained clearly beyond normal limits. This is in agreement with the observation made by Kumar et al. in conscious dogs.[10] It is of interest that with a substantial loss of left ventricular mass, cardiac function at rest returned to near pre-infarction levels, and that the elevation of LVEDP was the result of a reduction in left ventricular compliance.[11]

Pathologic examination of the myocardial infarctions which were produced revealed a wide variation in their size, despite the fact that the site of occlusion was fairly circumscribed around the mid LAD coronary artery, and despite the fact that these arteries were of quite similar size. Under these circumstances, the single most important determinant of infarct size from one animal to another seemed to be the unique anatomy of the coronary arterial



FIGURE 7.—Thirty-minute old thrombus. Epicardial aspect of artery is in upper left corner.

system, both of the occluded portion and of the adjacent unoccluded portions and the immediate availability of anastomotic collateral flow.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the skillful technical assistance of Miss Lilo Stucki, Mr. Nathaniel Lopey, Mr. Jose Rivera and Mr. Donald McNiece. The authors also wish to acknowledge the invaluable secretarial assistance of Miss Sally Evans, Mrs. Juliette Newman and Miss Margo Nikitas.

REFERENCES

1. Salazar, A. E. Experimental myocardial infarction: Induction of coronary thrombosis in the intact closed-chest dog. Circ. Res. 9:1351, 1961.
2. Rushmer, R. F., Finlayson, B. L., and Nash, A. A. Shrinkage of the heart in anesthetized, thoracotomized dogs. Circ. Res. 2:22, 1954.
3. Frank, M. J., and Levinson, G. E. An index of the contractile state of the myocardium in man. J. Clin. Invest. 47:1615, 1968.
4. Wegria, R., Segers, M., Keating, R. P., and Ward, H. P. Relationship between the reduction in coronary flow and the appearance of electrocardiographic changes. Amer. Heart J. 38:90, 1949.
5. Harris, A. S. Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. Circ. 1:1318, 1950.
6. Scherlag, B. J., Helfant, R. H., and Damato, A. N. The electrophysiological basis of ventricular arrhythmias induced by acute coronary ligation in dogs. Suppl. VI to Circ. 37/338:173, 1968 (Abstract).
7. Waldo, A. L., Kaiser, G. A., Castany, R. J., and Hoffman, B. F. A study of arrhythmias associated with acute myocardial infarction. Suppl. VI to Circ. 37/38:200, 1968 (Abstract).
8. Jorgensen, L., Rowsell, H. C., Hovig, T., and Mustard, J. F. Resolution and organization of platelet-rich mural thrombi in carotid arteries of swine. Amer. J. Patho. 51:681, 1967.
9. Shell, W. E., Kjekshus, J. K., and Sobel, B. E. Quantitative assessment of the extent of myocardial infarction in the conscious dog by means of analysis of serial changes in serum creatine phosphokinase activity. J. Clin. Invest. 50:2614, 1971.
10. Kumar, R., Hood, W. B., Joison, J., Jr., Norman, J. C., and Abelmann, W. H. Experimental myocardial infarction. II. Acute depression and subsequent recovery of left ventricular functions: serial measurements in intact conscious dogs. J. Clin. Invest. 49:55, 1970.
11. Bianco, J. A., Hood, W. B., Covelli, V. H., and Norman, J. G. Diminished ventricular compliance in experimental acute myocardial infarction. Suppl. VI to Circ. 37, VI-42, 1968 (Abstract).

DISCUSSION

H. L. Brooks, University of Chicago: My question is: In your rather inclusive study, did you do any mapping in terms of involvement of the septum, the right ventricular free wall and the left ventricular free wall in these LAD occlusions?

Dr. Hirsch: Yes we did. I should say that we found infarcts. Depending on whether we had gone straight down the LAD or off into a diagonal branch, if the thrombus was situated midway between apex and coronary osteum, we would find an infarct that involved the anterior wall or that extended around into the anterior part of the septum and might have involved also the apex as well. Sometimes it wrapped around and extended to the diaphragmatic surface of the apex. If we went into a diagnonal branch, off to the left, we would find an infarct that, more typically, involved the anterior wall and the free wall of the left ventricle. To make an all-inclusive statement: the most distal infarction boundary was the underside of the apex. The most medial boundary was the ventricular septum about half-way from the front to back. The most superior portion was where the thrombus was and that was never less than about 1 or 1½ centimeters below the bifurcation of the LAD from the circumflex. The most lateral involved a good deal of the free wall of the left ventricle.

P. Somani, Abbott Laboratories, North Chicago: Have you followed these dogs long enough to know what happens to the thrombus? Does it dissolve naturally or what is the progress? How long does it take?

Dr. Hirsch: Yes the thrombus evolves; it is in fact a quite dynamic structure. In the first three to four hours we would see lots of platelets and some fibrin. Within one to three days there would be much less platelet material, large amounts of fibrin, and within three to seven days you'd see infiltrates with inflammatory cells right through the thrombus. We didn't follow any dogs for more than eight days.

ANIMAL MODELS OF MYOCARDIAL INFARCTION: CURRENT LIMITATIONS AND FUTURE PROMISE

W. B. Hood, Jr. and J. C. Norman*

Our laboratories have been engaged for four years in developing myocardial infarction models in the dog, pig, and calf. Initially open-chest, acute occlusions of individual major coronary arteries were effected in all three species. Subsequently this methodology was extended to intact conscious animals utilizing single and multiple implantable coronary artery balloon-cuff occluders. With this approach discrete areas of the myocardium can be rendered temporarily ischemic or permanently infarcted. Approximately two hundred animals have been studied. Transient, acute occlusion of a major coronary artery with subsequent release within two minutes in the open-chest or awake, unanesthetized dog, pig or calf results in immediate, but reversible electrocardiographic changes of ischemia and hemodynamic alterations of moderate ventricular failure. Permanent occlusion of a major coronary artery in the awake, unanesthetized animal results in persistent and progressive electrocardiographic, hemodynamic and enzymatic alterations. Dogs develop significant hemodynamic changes, frequently without fatal dysrhythmias, whereas pigs and calves almost invariably fibrillate. No obvious pain reaction occurs in any of the species. Surviving dogs develop multifocal ventricular tachycardia in the period from 4 to 72 hours following permanent single vessel occlusion. Permanent multiple vessel occlusion is followed by progressive and unstable hemodynamic deterioration; such animals are not suitable for studies of steady-state cardiac failure. Thus far, no completely satisfactory model of stable, severe, persistent cardiac failure has been evolved. In the most suitable model, i.e., the dog, hemodynamic patterns of left ventricular failure are complicated by ventricular dysrhythmias. The absence of pain reactions in all three species in the awake state suggests inherent differences from clinical myocardial infarction. Promising areas of future study include the production of severe failure by the staged complete occlusion of the left anterior descending followed by partial occlusion of the circumflex coronary artery and observations on the pain-conditioned primate.

INTRODUCTION**

The recent resurgence of interest in the clinical entity of acute myocardial infarction has stimulated a search for improved models of acute coronary occlusion in animals. Models are being sought which will simulate the disease as it occurs in a clinical setting, which will prove suitable for evaluating the effects of medical therapy, and which will permit evaluation of newer methods of surgical intervention, including circulatory assist devices.

The methods which have heretofore been available for inducing acute myocardial infarction in animals have been manifold, and each possesses inherent advantages and disadvantages (Table I). To date, the most widely used methods have included direct ligation of a single large coronary vessel,[1] placement of ameroid constrictors about major coronary vessels,[2] and embolization of coronary vessels with microspheres.[3] The first method allows exact timing of infarction, but must be performed in the anesthetized open-chest animal, and has a considerable incidence of ventricular fibrillation. The second method also requires thoracotomy, but animals usually develop infarction after recovery from surgery, and the method is therefore suitable for chronic studies; the time of onset of infarction is unpredictable. The third method can be carried out in unoperated animals, but produces diffuse rather than focal myocardial damage, and therefore fails to simulate clinical myocardial infarction. Newer methods included insertion of a plug into a major coronary artery using a catheter-tipped device,[4] multiple ligation of small coronary vessels,[5] induction of coronary arterial thrombosis by a catheter tipped electrode,[6] embolization of the coronaries with metallic mercury,[7] and the induction of thrombosis by placing a magnesium

* Thorndike Memorial and Sears Surgical Laboratories and the Boston University Medical Service, Boston City Hospital, the Departments of Medicine and Surgery, Harvard Medical School, and the Department of Medicine, Boston University Medical School.

** Supported by NIH Grants PH 43-68-684, A0 71-2498, HE 5244, HE 10539, HE 14294, SF-57-111, Grant 71-1016 from the American Heart Association, the John A. Hartford Foundation, the John and Mary R. Markle Foundation, and the Charles E. Merrill Trust.

TABLE I.

Method	Thoracotomy Required	Unanesthetized Preparation	Focal Ischemia	Exact Timing of Infarction	"Trial" Occlusions Possible	Incidence Ventricular Fibrillation	Shock Model	Valid Serum Enzymes	Suitable for Chronic Studies
Direct ligation of a single large vessel[1]	+	0	+	+	0	variable	0	0	+
Ameroid constrictor[2]	+	+	+	0	0	?low	0	possibly	+
Microemboli[3]	0	0*	0	+	0	variable	+	+	?
Coronary "plug"[4]	0	0*	+	+	0	?high	0	+	?
Multiple ligation of small vessels[5]	+	0	+	+	0	?low	+	0	0
Electrothrombosis[6]	0	0*	+	+	0	?high	0	+	+
Mercury embolization[7]	0	0*	±	+	0	low	+	+	0
Metallic wire thrombosis[8]	0	+	+	0	0	?	0	+	+
Balloon cuff	+	+	+	+	+	low	0	+	+

* Anesthesia generally employed, but probably not mandatory.

alloy wire within a coronary artery through a catheter.[8]

The present report deals with the recently developed method of coronary occlusion which employs a balloon-cuff device implanted about the coronary artery.[9,10] Though thoracotomy is required, animals may be studied at any time after surgery. The exact timing of onset of infarction is possible. A unique feature of the method is the ability to carry out one or more "trial" occlusions. This permits studies of transient ischemia without permanent occlusion. The incidence of ventricular fibrillation is low, and the method is eminently suited to chronic studies of myocardial ischemia.

METHODS

Our studies have been carried out over the last four years in various animal species, including the dog, pig, calf and baboon. Initially, extensive testing of the balloon cuff occlusion device was carried out in open-chest anesthetized animals.[11,12,13] These studies demonstrated the feasibility and consistency of coronary occlusion with this device. Subsequently this methodology was extended to intact conscious animals utilizing single and multiple implantable coronary artery balloon occluders. The method entails implantation of the balloon-cuff occluders on the left anterior descending or circumflex coronary arteries or both (Figure 1). The animals were allowed to recover from surgery for approximately two weeks. At the time of study the animals were sedated using 0.8 mg/kg of morphine sulfate given I.M. for dogs, 2–3 mg/kg of morphine sulfate given I.M. for calves, and 1 mg of morphine sulfate given I.M. for pigs. Pigs were difficult to tranquilize using this regimen, and on occasion required 1 mg/kg of phencyclidine or more to produce muscle relaxation. For hemodynamic studies catheters were inserted acutely under local anesthesia. Cardiac output measurements were performed using indocyanine green dye as indicator.*

* Kindly supplied by Hynson, Westcott, and Dunning, Baltimore, Md.

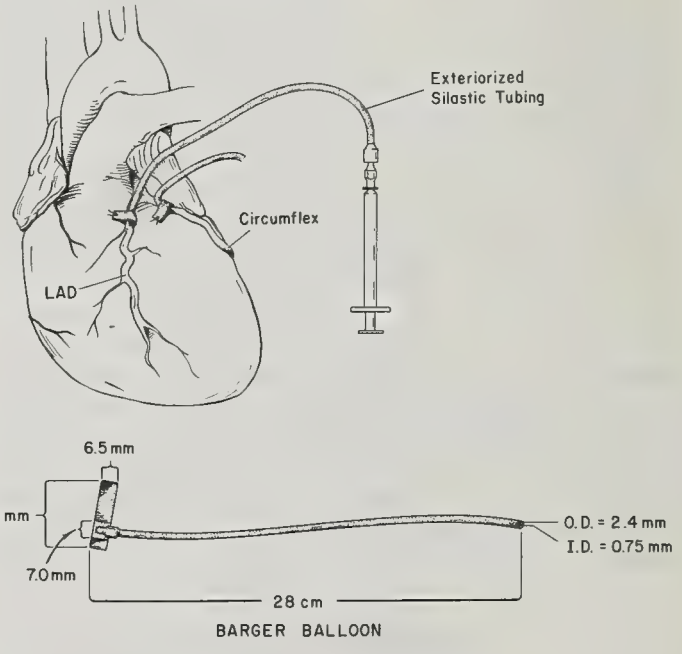


FIGURE 1.—Above: Schematic representation of balloon cuffs implanted on the left anterior descending and circumflex coronary arteries of a dog. Balloon is inflated through exteriorized Silastic tubing.
Below: Dimensions of balloon cuff and tubing.

Prior to permanent vessel occlusion, one or more "trial" occlusions, using an increasing volume of fluid in the balloon, is employed to ascertain the volume required for ischemia to occur. Subsequently, permanent occlusion is carried out, resulting in myocardial infarction. In our initial studies, permanent inflation of a single coronary artery balloon cuff was performed over a period of one-half hour or more using a microinfusion pump.[14] It was subsequently determined that occlusion may be safely carried out over a period of ten to fifteen minutes by manual inflation of the balloon cuff without an untoward incidence of ventricular fibrillation, and this method is now used exclusively. Onset of myocardial infarction may be determined by electrocardiographic and hemodynamic changes (vide infra), and subsequently by a rise in serum enzymes (Figure 2).

RESULTS OF STUDIES IN VARIOUS SPECIES

Approximately two hundred studies in various animal species have been carried out, the majority in dogs.

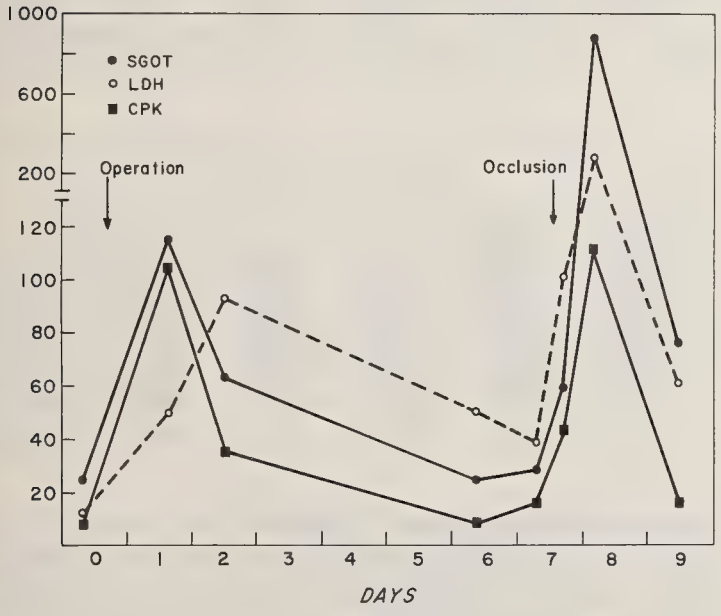


FIGURE 2.—Serial serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) levels in one dog. Following balloon implantation (operation) there was an initial increase in enzymes, which returned to normal over the next week. Subsequently a second higher enzyme rise resulted from balloon cuff coronary occlusion. (By permission.[14])

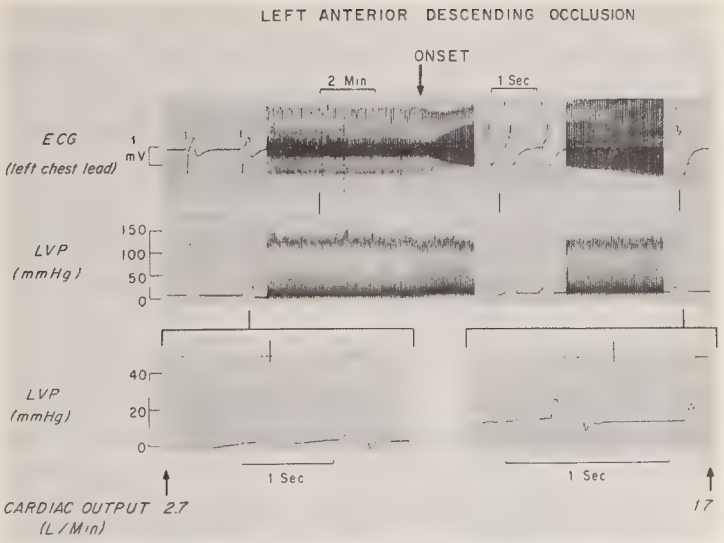


FIGURE 3.—Electrocardiographic and hemodynamic measurements in a dog during left anterior descending coronary artery occlusion. Above: At the onset of occlusion, there is initial peaking of T waves, followed within a few minutes by ST segment elevation. Below: High-gain left ventricular pressure (LVP) tracings show an increase in left ventricular end-diastolic pressure with occlusion. There is a decline in cardiac output, but little change in left ventricular systolic pressure. (By permission.[15])

Canine Studies

Approximately 180 studies have been carried out in intact conscious dogs. A large body of evidence has been gathered which shows that consistent electrocardiographic and hemodynamic changes occur in this species (Figure 3). Typically, with left anterior descending coronary occlusion, the electrocardiogram shows initial peaking of the T waves in the left anterior chest leads followed within several minutes by elevation of the S-T segment. The S-T segment often regresses toward normal over a one-hour period following myocardial infarction. From 5 to 30 minutes after onset of coronary occlusion dogs often show ventricular irritability, and on occasion this degenerates into ventricular fibrillation. However, the incidence of ventricular fibrillation within the first few hours after myocardial infarction in this preparation is low, approximating 20%.[15] From 4–72 hours after myocardial infarction dogs typically develop a multifocal ventricular tachycardia, during which another small percentage of animals may succumb to ventricular fibrillation. Thereafter the animals return to normal sinus rhythm, and survival is virtually assured.

Hemodynamic changes of mild to moderate left ventricular failure also occur quite consistently, and these consist of a rise in heart rate to sinus tachycardia levels, and a rise in left ventricular end-diastolic pressure (Table II). There is a modest decline in cardiac output in many animals, but it is not severely depressed. Arterial blood pressure is well maintained and declines only slightly.[14]

TABLE II.—*Left Anterior Descending Coronary Artery Occlusion in 15 Intact Conscious Dogs (Mean ± SEM)*

	Left Ventricular Peak Systolic Pressure (mm Hg)	Heart Rate (beats/min)	Left Ventricular End-Diastolic Pressure (mm Hg)	Cardiac Output (L/min)
Control	156±6	82±5	6.8±0.8	3.5±0.3
Occlusion	142±7*	132±9*	19.2±1.8*	2.8±0.3*

* $p < 0.05$, paired differences

We have carried out extensive studies of the natural course of this left ventricular failure in dogs following acute myocardial infarction, and we have also studied the effects of various interventions. Improvement in left ventricular performance is noted in the healing phase one week after myocardial infarction, as indicated by return of the elevated heart rate and the depressed cardiac output towards normal, and also by improvement of initially depressed ventricular function curves.[16] Elevation of left ventricular end-diastolic pressure in the healing phase is likely to persist and this has been attributed to residual left ventricular failure, but also to reduced compliance of the infarcted ventricle.[17]

In this preparation various inotropic agents have been tested, including digitalis in the form of acetyl strophanthidin,[18] glucagon,[19] rapid atrial pacing,[20] and phentolamine.[21] With one exception, these interventions were found to improve ventricular performance at various stages after infarction, manifested by a rise in cardiac output and lowering of left ventricular end-diastolic pressure. However, when acetyl strophanthidin is infused in the acute phase of infarction, such changes do not occur, and this appears to be due to a systemic vasoconstrictor effect of digitalis and an attendant increase in afterload to contraction (Figure 4). Glucagon, on the other hand, typically raises cardiac output and lowers left ventricular end-diastolic pressure in both the acute and healing phases of infarction (Figure 5). Similar results are obtained with phentolamine, an agent which is classically known as an alpha-blocking agent, but which in addition has inotropic effects. Rapid atrial pacing also improves left ventricular performance in a similar fashion, implying either that the inotropic effect of the rapid rate is beneficial to the infarcted ventricle, or that the intrinsic heart rate response to infarction is inadquate to compensate for the diminished stroke volume caused by the ischemic lesion.

It has also proven possible in this preparation to place indwelling coronary sinus catheters under fluoroscopy and to study myocardial ener-

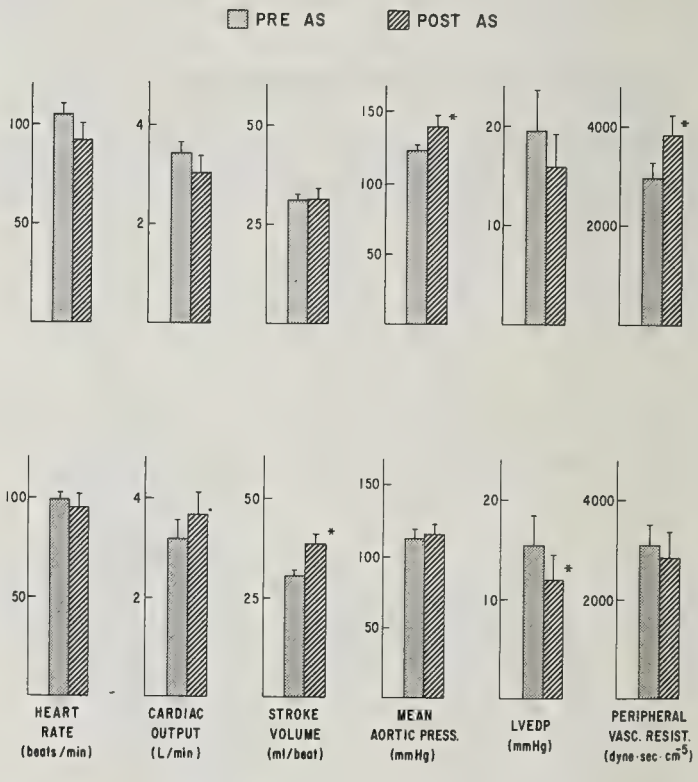


FIGURE 4.—Hemodynamic effects of infusing 3 μg/Kg/min of acetyl strophanthidin in 6 dogs one hour (above) and one week (below) after coronary occlusion. Above: In the acute phase of infarction acetyl strophanthidin caused an increase in aortic mean pressure and peripheral vascular resistance, but no other significant changes. Below: In the healing phase of infarction acetyl strophanthidin caused an increase in stroke volume and decline in left ventricular end-diastolic pressure, evidence of improved ventricular performance. The pressor effect was absent. Data shown as mean ± SEM.
*$p < 0.05$, paired differences.

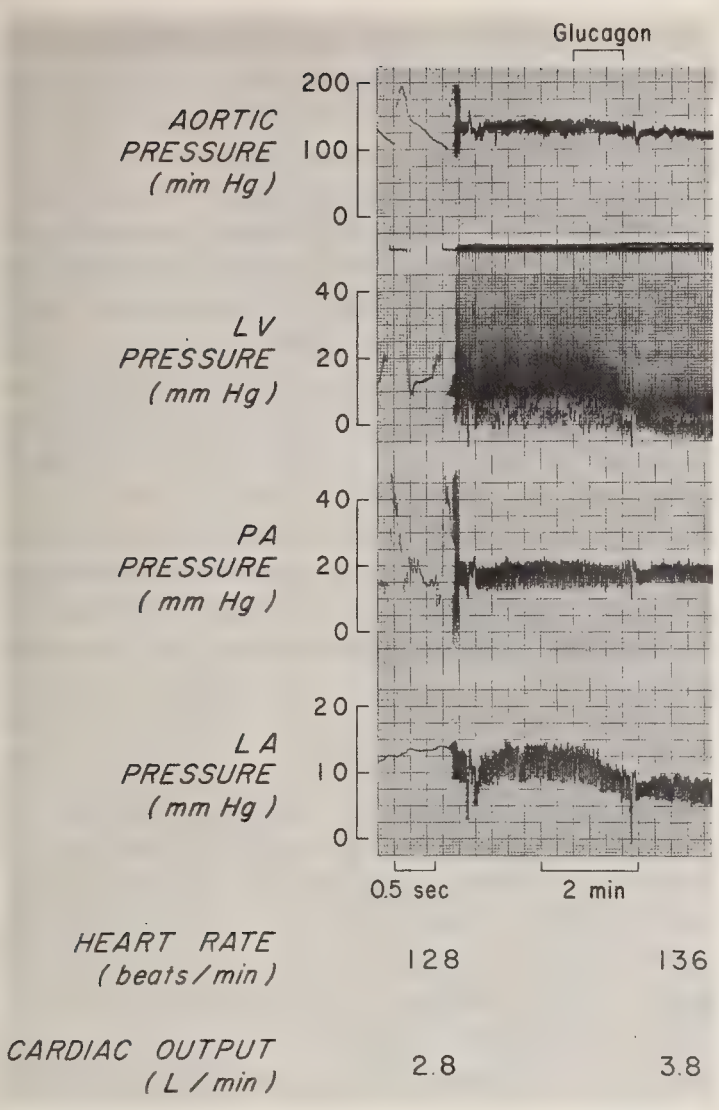


FIGURE 5.—Tracings obtained during infusion of 50μg/Kg of glucagon into the pulmonary artery of an intact conscious dog one week after acute myocardial infarction. There is a decline in the initially elevated left atrial (LA) pressure, and an increase in heart rate and cardiac output following glucagon infusion. Similar results were observed after glucagon infusion in the acute phase of infarction. LV = Left ventricular. PA = Pulmonary arterial. (By permission.[19])

getics. In one study,[19] the observation was made that glucagon-induced inotropy after infarction was not accompanied by an increase in myocardial oxygen consumption (Figure 6). Presumably this was due to the coincident lowering of left ventricular end-diastolic pressure and volume, resulting in diminished wall stress and myocardial oxygen requirement, thus counterbalancing the increased oxygen requirement of increased inotropy. These observations are of interest in view of the current debate concerning the role that inotropic agents may play in increasing myocardial oxygen consumption and increasing damage in acutely infarcted myocardium.[22] Evidently, this latter phenomenon may not exist when left ventricular failure is present and when the inotropic agent simultaneously reduces ventricular filling pressure and volume.

This canine preparation has also been tested with one intervention directed toward the problem of salvaging myocardial cells around the margins of the infarct from ischemic damage, namely, hyperbaric oxygenation.[23] This intervention was found to be without effect upon hemodynamic abnormalities even when applied at a time when ischemic damage was potentially reversible. The preparation seems to be suitable for testing other such interventions, including

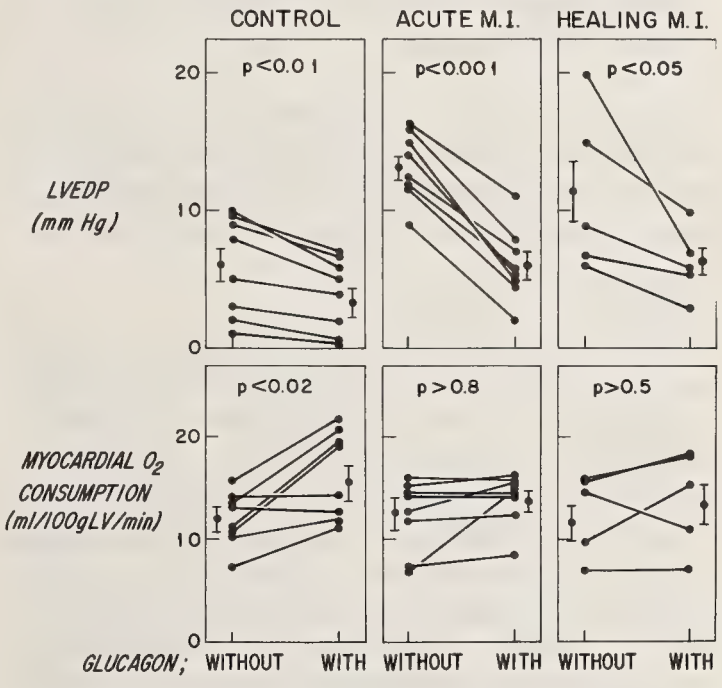


FIGURE 6.—Changes in left ventricular end-diastolic pressure (LVEDP) and myocardial oxygen consumption in unanesthetized dogs studied in the control state and one hour after myocardial infarction (acute MI-8 dogs), and again one week later (healing MI-5 dogs). Values before and 13 minutes after administration of 50 μg/Kg of glucagon are shown. Bars show mean ± SEM. In the control state LVEDP declined slightly but significantly after glucagon; oxygen consumption increased significantly. In acute and healing MI LVEDP declined substantially from the initially elevated values, while myocardial oxygen consumption did not increase significantly. See text for discussion. (By permission.[19])

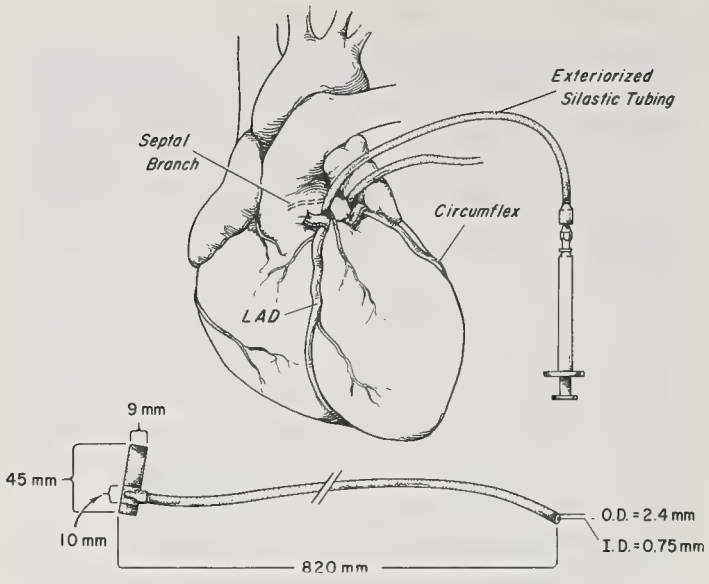


FIGURE 7.—Above: Schematic representation of balloon cuffs implanted on the left anterior descending and circumflex coronary arteries of a calf.
Below: Dimensions of balloon cuff and tubing. (By permission.[13])

infusion of various substrates or induction of hypothermia, since a well defined endpoint of hemodynamic improvement or deterioration is present. Such studies remain to be carried out.

Studies in Calves

The method of balloon cuff coronary occlusion has been studied in seven intact conscious calves.[13] The technique employed is similar to that used for canine studies except that a larger balloon device is employed to accommodate the larger coronary arteries in the calf (Figure 7). Hemodynamic changes indicative of mild to moderate left ventricular failure were also produced by balloon cuff coronary occlusion (Table III). It is noteworthy that, despite hemodynamic changes comparable to those occurring in dogs, calves invariably develop ventricular fibrillation in the first hour after major coronary occlusion, and that the rapidity of onset of ventricular fibrillation appears to be related to the severity of failure (Figure 8).

TABLE III.—*Left Anterior Descending Coronary Artery Occlusion in Seven Intact Conscious Calves (mean ± SEM)*

	Aortic Mean Pressure (mm Hg)	Heart Rate (beats/min)	Left Ventricular End-Diastolic Pressure (mm Hg)	Cardiac Output (L/min)
Control	129± 8	103±10	7.6±1.6	5.2±0.7
Occlusion	131±10	112± 8	15.7±4.4*	4.4±0.6*

* $p < 0.05$, paired differences

Studies in Pigs

Studies have been carried out in seven intact conscious pigs. In general these animals are more difficult to work with than either dogs or calves, and often require heavy sedation. Hemodynamic studies are incomplete in this species; however, electrocardiographic monitoring has been carried out. Electrocardiographic changes of ischemia appear to be less marked in pigs than in dogs, and the incidence of ventricular fibrillation is high (four of seven animals). Because of the similarity of the porcine coronary circulation to man, further studies in this somewhat more difficult preparation may be indicated.

General Comments

The desirability of carrying out studies of circulatory physiology in unanesthetized as opposed to anesthetized animals is established, and this may apply particularly to studies of myocardial ischemia.[24] Acute myocardial infarction in unanesthetized animals is characterized by sinus tachycardia and a rise in left ventricular end-diastolic pressure. On the other

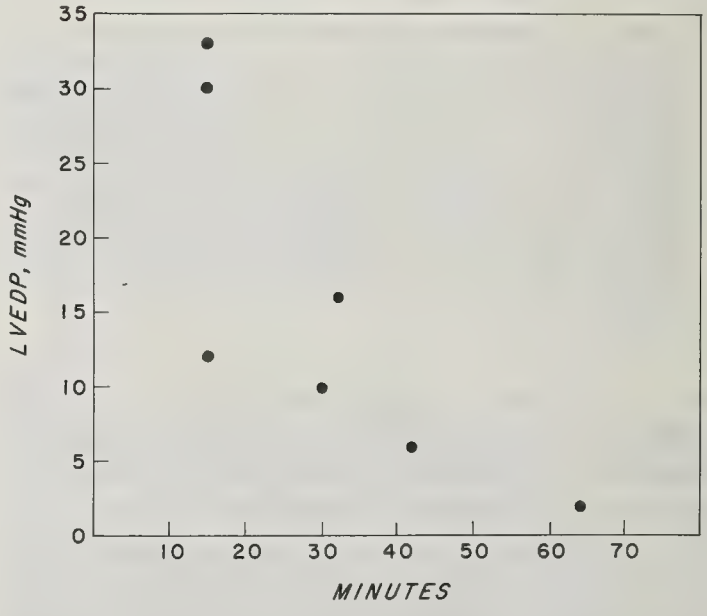


FIGURE 8.—Height of left ventricular end-diastolic pressure plotted in relation to time of onset of ventricular fibrillation following occlusion of the left anterior descending coronary artery in calves. (By permission.[13])

hand, studies in anesthetized animals do not show marked alterations in heart rate, left ventricular filling pressures are seldom markedly elevated, and hypotension and cardiogenic shock appeared to be potentiated. It seems clear that further studies of myocardial ischemia which are designed to simulate clinical situations should utilize unanesthetized animals whenever this is feasible.

GRADED VENTRICULAR FAILURE FROM OBSTRUCTION OF MULTIPLE CORONARY VESSELS

Whereas obstruction of a single major coronary vessel in the dog usually results in mild to moderate left ventricular failure, complete occlusion of both the left anterior descending and circumflex vessels leads to marked cardiac decompensation (Figure 9). Such severe ischemia cannot be long maintained without the demise of the animal. Extensive studies carried out in 18 dogs have shown, however, that it is possible to produce total obstruction of one major coronary vessel and a partial stenosis of another, resulting in stable states of left ventricular failure.[25] In the majority of these animals, anterior wall myocardial infarction was produced by occlusion of the left anterior descending coronary artery, the animals were allowed to recover for one or more weeks, and then partial obstruction of the circumflex coronary vessel was carried out. The degree of obstruction of the circumflex vessel is highly critical, and it is difficult to obtain a stable state of left ventricular decompensation from manual inflation of the circumflex balloon.

Several methods have been tested in an attempt to stabilize the preparation. By monitoring the pressure in the circumflex balloon, and holding this pressure constant, it is possible to produce left ventricular failure which is stable for many minutes (Figure 10). Recent testing with a more sophisticated method has also been carried out in our laboratory in three dogs. In this method the left atrial pressure is monitored and utilized to drive a servo-controlled pump-motor combination, which infuses or withdraws minute amounts of fluid from the circumflex balloon to hold left atrial pressure constant at a preselected level. Using this method it has been possible to stabilize left atrial pressure at a desired value in a range of 20–30 mm Hg for many hours.[26]

Of the total of 18 dogs studied with severe coronary ischemia, four have developed acute pulmonary edema, indicating the severity of the left ventricular ischemic lesion. In the future, more studies should be directed toward severe failure preparations. Present studies by many investigators often deal with preparations in which the degree of ischemic ventricular damage is slight, thus failing to simulate the clinical situation in which interventions are most likely to be required. An ideal preparation of severe left ventricular failure does not yet exist, however, the servo-controlled method described above appears to hold considerable promise.

We have not carried out studies of multiple vessel coronary occlusions in intact unanesthetized calves or pigs. However, studies of transient double-vessel coronary occlusion in eleven anesthetized calves suggest that results similar to those in dogs would be obtained.[13] The extensive studies of LaFarge and Bernhard and coworkers utilizing left main coronary artery occlusion in intact unanesthetized calves indi-

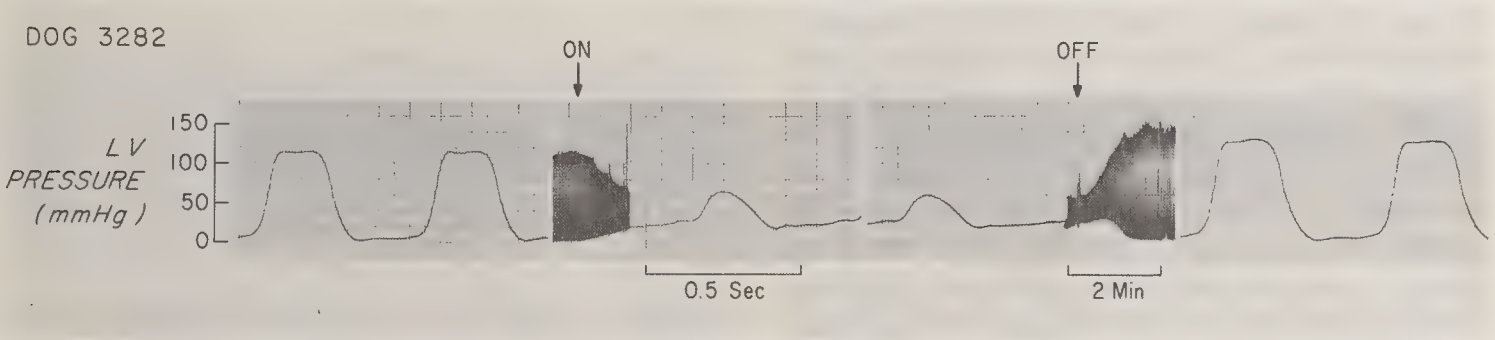


FIGURE 9.—Left ventricular pressure in a dog during transient occlusion of both the left anterior descending and circumflex coronary arteries. There is severe and progressive deterioration in left ventricular performance, as shown by the marked fall in systolic and rise in diastolic pressures. (By permission.[25])

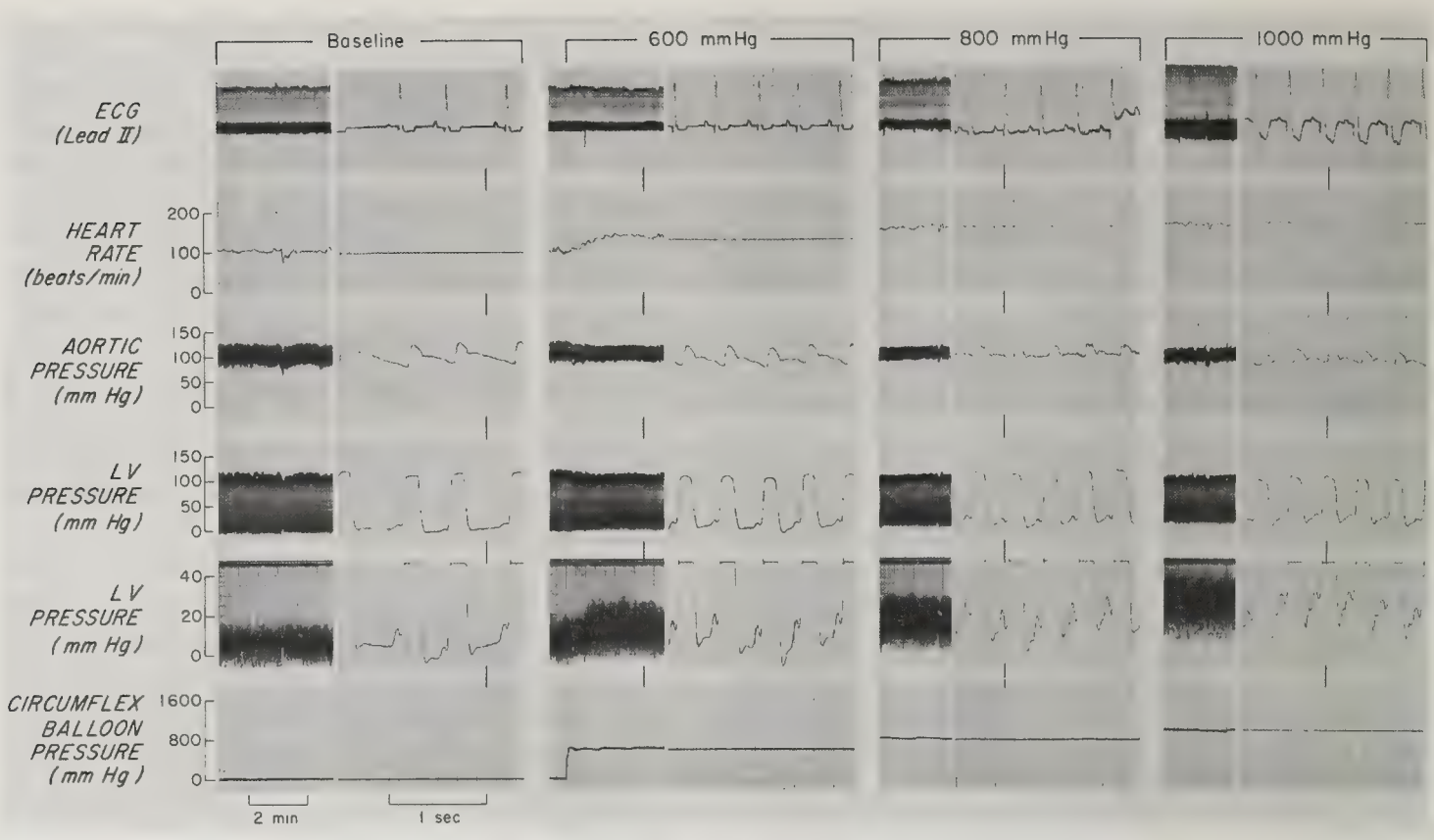


FIGURE 10.—Stable left ventricular failure produced by total occlusion of the left anterior descending plus graded constriction of the circumflex coronary artery in a dog. Control of the degree of circumflex constriction was achieved by monitoring and holding constant the pressure in the implanted circumflex balloon. Increasing severity of left ventricular failure is evident from the rise in heart rate and left ventricular end-diastolic pressure as circumflex balloon pressure is successively increased to 600, 800, and 1000 mm Hg. (By permission.[25])

cate that marked left ventricular decompensation does occur.[27]

OBSERVATIONS ON THE OCCURRENCE OF PAIN REACTIONS IN ANIMAL STUDIES OF MYOCARDIAL ISCHEMIA

Studies of acute myocardial infarction in dogs, pigs and calves are notable for the lack of any apparent pain reaction or discomfort on the part of the animal. Dogs sometimes appear to become slightly restless several minutes after total and permanent coronary occlusion, but this is variable, and is not influenced by the presence or absence of morphine pretreatment.[14] Whether this lack of pain reaction represents a species difference from man, the effect of sedation, the effect of vessel dissection and interruption of nerve supply to the heart during placement of the coronary balloon occluding cuff, or some other yet undiscovered cause remains unknown. It may be appropriate to study a species more closely related to man, in which it might be possible to condition animals to give an indication whether pain is present or absent. We have recently initiated studies in baboons with implanted coronary artery balloon occlusion cuffs.[28] Testing in one animal showed that typical electrocardiographic coronary ischemic patterns are obtained (Figure 11). Thus far, only transient one-minute occlu-

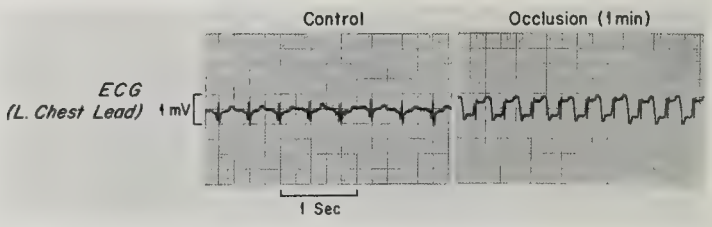


FIGURE 11.—Left: Resting electrocardiogram obtained in an unanesthetized baboon. Right: Tracing obtained one minute after left anterior descending coronary artery occlusion, showing marked elevation of ST segments.

sions have been carried out. During this period the animal gave no evidence of discomfort or a painful reaction. This method deserves further exploration in the future, and if pain reactions are obtained, the preparation will be eminently suitable for studying the total clinical spectrum of acute coronary occlusion in an animal model which closely simulates the syndrome in man.

REFERENCES

1. Harris, A. S. Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. Circulation 1:1318–1328, 1950.
2. Vineberg, A., Mahanti, B., and Litvak, J. Experimental gradual coronary artery constriction by ameroid constrictors. Surgery 47:765–771, 1960.
3. Agress, C. M., Rosenberg, M. J., Jacobs, H. I., Binder, M. J., Schneiderman, A., and Clark, W. G. Protracted shock in the closed-chest dog following coronary embolization with graded microspheres. Amer. J. Physiol. 170:536–549, 1952.
4. Hammer, J., and Pisa, Z. A method of isolated, gradual occlusion of a main branch of a coronary artery in closed-chest dogs. Amer. Heart J. 64:67–70, 1962.
5. Goldfarb, D., and Gott, V. L. Cardiovascular alterations during declining and steady-state low cardiac output secondary to coronary insufficiency in the dog. J. Thorac. Cardiovasc. Surg. 56:578–589, 1968.
6. Weisse, A. B., Lehan, P. H., Ettinger, P. O., Moschos, C. B., and Regan, T. J. The fate of experimentally induced coronary artery thrombosis. Am. J. Cardiol. 23:229–237, 1969.
7. Lluch, S., Moguilevsky, H. C., Pietra, G., Shafer, A. B., Hirsch, L. J., and Fishman, A. P. A reproducible model of cardiogenic shock in the dog. Circulation 39:205–218, 1969.
8. Kezdi, P., Marshall, W. J., Jr., and Stanley, E. L. Hemodynamic and biochemical measurements in experimental myocardial infarction. Am. J. Cardiol. 19:135, 1967.
9. Chimoskey, J. E., Szentivanyi, M., Zakheim, R., and Barger, A. C. Temporary coronary occlusion in conscious dogs: Collateral flow and electrocardiogram. Am. J. Physiol. 212:1025–1032, 1967.
10. Khouri, E. M., Gregg, D. E., and Lowensohn, H. S. Flow in the major branches of the left coronary artery during experimental coronary insufficiency in the unanesthetized dog. Circ. Res. 23:99–109, 1968.
11. Hood, W. B., Jr., Covelli, V. H., Abelmann, W. H., and Norman, J. C. Persistence of contractile behavior in acutely ischemic myocardium. Cardiovasc. Res. 3:249–260, 1969.
12. Hood, W. B., Jr., Covelli, V. H., and Norman, J. C. Acute coronary occlusion in pigs: Effects of acetyl strophanthidin. Cardiovasc. Res. 3:441–446, 1969.
13. Molokhia, F. A., Asimacopoulos, P. J., Kumar, R., Hood, W. B., Jr., and Norman, J. C. Controlled production of left ventricular failure in intact calves using implanted coronary occluders. Ann. Thor. Surg. 10; 503–511, 1970.
14. Hood, W. B., Jr., Joison, J., Kumar, R., Katayama, I., Neiman, R. S., and Norman, J. C. Experimental myocardial infarction. I. Production of left ventricular failure by gradual coronary occlusion in intact conscious dogs. Cardiovasc. Res. 4:73–83, 1970.
15. Joison, J., Kumar, R., Hood, W. B., Jr., and Norman, J. C. An implantable system for producing left ventricular failure for circulatory assist device evaluation. Trans. Amer. Soc. Art. Int. Organs 15:417–422, 1969.
16. Kumar, R., Hood, W. B., Jr., Joison, J., Norman, J. C., and Abelmann, W. H. Experimental myocardial infarction. II. Acute depression and subsequent recovery of left ventricular function: Serial measurements in intact conscious dogs. J. Clin. Invest. 49:55–62, 1970.
17. Hood, W. B., Jr., Bianco, J. A., Kumar, R., and Whiting, R. B. Experimental myocardial infarction. IV. Reduction of left ventricular compliance in the healing phase. J. Clin. Invest. 49:1316–1323, 1970.
18. Kumar, R., Hood, W. B., Jr., Joison, J., Gilmour, D. P., Norman, J. C., and Abelmann, W. H. Experimental myocardial infarction. VI. Efficacy and toxicity of digitalis in acute and healing phase in intact conscious dogs. J. Clin. Invest. 49:358–364, 1970.
19. Kumar, R., Sharma, G.V.R.K., Molokhia, F. A., Norman, J. C., Inamdar, A. N., Messer, J. V., Abelmann, W. H., and Hood, W. B., Jr. Experimental myocardial infarction. X. Efficacy of glucagon in acute and healing phase in intact conscious dogs: Effects on hemodynamics and myocardial oxygen consumption. Circulation 45:55–64, 1972.
20. Kumar, R., Joison, J., Gilmour, D. P., Molokhia, F. A., Pegg, C. A. S., and Hood, W. B., Jr. Experimental myocardial infarction. VIII. Chronotropic augmentation of cardiac function in left ventricular failure of acute and healing stages in intact conscious dogs. J. Clin. Invest. 50:217–225, 1971.
21. Hood, W. B., Jr., Singh, J. B., Abelmann, W. H., and Polansky, B. J. Positive inotropic effects of phentolamine in left ventricular failure due to experimental myocardial infarction. Circulation 34 (Supp. II) : 127, 1971.
22. Hood, W. B., Jr. Salvage of myocardium in acute ischemia. Circulation 43:11–13, 1971.
23. Gilmour, D. P., Hood, W. B., Jr., Kumar, R., Joison, J., Norman, J. C., and Abelmann, W. H. Hyperbaric oxygenation in acute myocardial infarction in intact conscious dogs. Fed. Proc. 29:586, 1970.

24. KUMAR, R., HOOD, W. B., JR., and ABELMANN, W. H. Hemodynamic spectrum of left ventricular failure in experimental myocardial infarction. Amer. Heart J. 82:713–714, 1971.
25. HOOD, W. B., JR., KUMAR, R., JOISON, J., and NORMAN, J. C. Experimental myocardial infarction. V. Reaction to impaired circumflex flow in the presence of established anterior myocardial infarction in intact conscious dogs. Amer. J. Cardiol. 26:355–364, 1970.
26. WAGNER, R., HOWLAND, P., HOOD, W. B., JR., and ABELMANN, W. H. A servo-controlled, canine model of stable severe ischemic left ventricular failure. Fed. Proc. 31: 330 Abs, 1972.
27. BERNHARD, W. F., LAFARGE, C. G., HUSIAN, M., YAMAMURA, N., and ROBINSON, T. C. Physiological observations during partial and total left heart bypass. J. Thorac. Cardiovasc. Surg. 60:807–819, 1970.
28. NORMAN, J. C., MOLOKHIA, F. A., and HOOD, W. B., JR. Primate models of ischemic left ventricular failure: Absence of pain reaction with transient coronary occlusion. Fed. Proc. 31: 628 Abs, 1972.

DISCUSSION

CHAIRMAN LA FARGE: Thank you very much, Dr. Hood, for a beautiful presentation of a multi-species approach to the age-old problem of coronary occlusion.

FRANCIS L. EARL, Food and Drug Adm., Washington, D.C.: You made a statement that swine are hard to work with. In what way did you find them hard to work with?

DR. HOOD: Yes; I should clarify our methods for studying these animals. To date, none of our studies have been done in free-ranging animals. Our catheters are acutely inserted under local anesthesia and removed at the end of the study; then reinserted for restudies. We are working with a restrained animal, lying on its side, rather than with an unrestrained or otherwise free-ranging animal. We have found it very difficult to manage Chester White pigs under these conditions, even under sedation with various drugs. Is this different from your experience?

DR. EARL: This is a deficiency of your technique. I mean: they can be handled. With your ingenuity, you should be able to come up with a method for handling them. It is not very difficult.

CHAIRMAN: May I make a brief comment? A number of us have worked with pigs and had perhaps more difficulties with that species than with others. We've had relatively good results when working with pigs. They tend to behave like dogs, i.e., they are quite trainable, they are free-ranging, they eat their own ECG leads and anything else that's attached to them; they are quite nice when they are trained, but they do present problems which are similar to those presented by the dog, except for the clotting area.

M. R. MALINOW, Beaverton, Oregon: I would like to add that you have to have a lot of ingenuity to work with baboons. They not only eat the leads, but also the hands of the experimenters.

CHAIRMAN: I'd like to ask a question in that line which has been raised as one of the topics of this whole conference: it is the question of intrinsic diseases in animals and animal colonies. You had a chance to observe several animals in a colony. Would you like to comment on their behavior, in response to Dr. Malinow, and on the intrinsic management of the disease, particularly in baboons?

DR. HOOD: Our experience with baboons is still limited at this point. One of the obvious problems in all of our dog experiments was the spontaneous occurrence of pneumonia which is a real problem in chronic animal studies. Our studies have at times been hampered, particularly in the winter season, by pneumonia. We have not in this rather large series of 180 come across spontaneous abnormalities of the circulatory system or congenital anomalies that have limited the study and have not had to discard animals on this basis.

R. E. GOLDSTEIN, NIH, Bethesda, Md.: My question is how do you decide where to place the balloon cuffs.

DR. HOOD: I think the placement of balloon cuffs is an important problem. One would wish that there were some way we could assess in advance where the animal should have its balloon cuffs placed in order to develop just the right amount of failure without going into ventricular fibrillation, which is clearly related to the extent of the ischemic damage. Our practice has been to place the cuffs at the tip of the left atrial appendage in the dog and in the other species, usually beyond the first marginal

branch. But we do try to make some assessment of the overall size and distribution, by inspecting the apex of the heart, of the left anterior descending coronary in the dog and in other animals, to see whether this is an oversized or an undersized left anterior descending vessel. If it is undersized, we will move the occluding cuff up just below the septal artery to assure an adequate size infarct.

J. C. NORMAN, Boston City Hospital, Boston, Mass.: In terms of tape, we've got about five hundred tapes in the lab; of baboons, cows, calves and dogs, I think the trustees of the hospital know that the dogs are there. They're not aware of the other animals. We've had a rather extensive experience in this particular area and in other areas; in terms of expensive instrumentation, it can somehow disappear. The point I would like to make in this particular presentation—and I'm sure Dr. Hood will have other comments—is that this is an old technique. We first developed it in terms of working on intermittent claudication as residents years ago. It opens up so very much in terms of gathering physiologic data on something that is clinically pertinent to us in the wards all the time. The conversations between Dr. Sawyer, Dr. LaFarge, and Dr. Garcia who runs the primate center and is present here, regarding the questions of clinical relevancy has been simply a matter of moving back and forth from the Intensive Care Unit down to the laboratory. We think that there is a great deal to be done yet. It is a relatively simple preparation. We, as Dr. Hood's animal caretakers and surgeons, can prepare the animals. He, of course, is the mind behind all this. Summing up: we enjoyed the work and hope to continue it for a long time.

CHAIRMAN: Thank you Dr. Norman. Dr. Weber?

DR. K. T. WEBER, JR., University of Alabama, Birmingham Medical Center, Birmingham, Alabama: We had some experience with calves, using vascular occluders and I'd like to echo the comment about the 100% mortality with fatal ventricular arrhythmias. However, we were recently much encouraged by the use of massive doses of regular insulin which were not only able to control the frequency of PVCs, but when injected locally into the coronary vessels distal from the ligation, was actually able to reverse the ventricular tachycardia. We do not know the mechanism involved here and we're pursuing the problem at this point. I believe, however, that it might be of interest for those who are working with calves and possibly for those working with dogs.

DR. NORMAN: I've wondered if this fact about VF in the calf and pig might be related in some way to the relatively end-artery structure of the calf and pig, as compared to the dog. Your observations on the use of insulin are interesting. Do you give potassium glucose with this?

DR. WEBER: Yes sir, we do. We give 10% glucose and intravenous potassium to prophylactically prevent complications. We thought that Hyatt possibly reported the same observation in dogs last year in cardiovascular research and postulated the mechanism of epinephrine blockade. We were unable to block epinephrine, glucagon, or isoproteronal effects, inotropic or chronotropic, with massive doses of insulin. We believe at this point that it may be a metabolic effect, stabilizing membranes, preventing electric chemical gradients and the like. We are not sure.

CHAIRMAN: Again, I'd like to refer to the aricle by Alexander Leaf at Massachusetts General, discussing ischemic changes: he referred to them in an article which, unfortunately, I can't cite for you. The study involved rabbits. The changes we were discussing earlier, in terms of opening pressure, revascularization or better vascularization of what's already there, were stimulated by the use of either glucose or glucose-insulin combinations. The presence of the insulin was not the critical factor. The changes were also stimulated by hyperosmolar concentrations of mannitol. What this was really was a study in cerebral ischemia, the improvement and maintenance of circulation in the brain and profusion of the brain were markedly improved both by glucose, by mannitol, by prophylactic carbon dioxide infusion and by a combination of all three. Accordingly, there certainly is a substrate effect, probably from the glucose, and a hyperosmolar effect from substances like mannitol.

TURKEY ROUND HEART DISEASE: A MODEL CARDIOMYOPATHY

E. F. Jankus,* N. A. Staley** and G. R. Noren***

Turkey Round Heart Disease (RH) is characterized by cardiomegaly in the growing poult. An experimental breeding flock has been established with progeny having a grossly identified incidence of RH of up to 26 percent. Breeding trials suggest that the disease has a familial mode of inheritance. In all birds examined histologically, a wide spectrum of focal myocarditis is evident within the first week after hatching, and increases in severity to two weeks of age. Some birds that survive this acute phase develop biventricular dilatation at two to three weeks of age. The RH male to female death ratio during the chronic phase is approximately two to one. Left ventricular endocardial fibroelastosis becomes prominent after one month of life, and progresses in severity thereafter. The clinical diagnosis of RH after the acute phase is based upon electrocardiographic (ECG) evidence of a shift in the frontal plane axis of the QRS complex. Approximately 30% of five week old RH turkeys have cardiac murmurs consistent with mitral insufficiency. ECG evidence of spontaneous RH recovery is associated with conversion to a normal QRS axis, but with significantly lower spatial vector strength. Recovery during the chronic phase occurs in about one-half of the RH birds previously diagnosed. Hemodynamically, both the ECG identified RH and recovered birds are hypotensive, and have increased cardiac filling pressures. Features of turkey RH are consistent with J. F. Goodwin's [LANCET 1 (1970) 731] classification of a congestive cardiomyopathy.

INTRODUCTION

Round Heart Disease (RH) in turkeys has been described by several authors.[1-5] It has been defined as having acute and chronic phases.[4] The acute phase occurs during the first two weeks of life and is characterized by pancarditis, focal interstitial myocarditis, and high mortality. A wide spectrum of cardiac dilatation, ventricular hypertrophy and left ventricular endocardial fibroelastosis (EFE) characterizes the chronic phase, while death during this phase is associated with congestive heart failure. The criteria for electrocardiographic (ECG) identification of RH turkeys have been reported.[6,7]

This report will present data of breeding trials on the mode of inheritance of turkey RH and the clinical course of the disease during the chronic phase.

METHODS

Breeding Trials

Two blood lines, Flocks 1 and 2, of small broad breasted white turkeys with known past experience of RH were used for breeding experiments (1971). All hens were artificially inseminated. "Pooled" semen from toms of the respective flocks was used for insemination of the pen mated hens. From Flock 2 sixteen hens and six toms were selected for pedigreeing. Eggs from all groups were set at weekly intervals, and fertility was determined by candling on the fourteenth day of incubation. Hatchability was calculated on the basis of number of fertile eggs. Hatched poults were fed a commercial "starter" ration until five weeks of age. At this time those poults not saved for breeder replacement were sacrificed. The presence or absence of RH in the replacement poults was determined by ECG.

Clinical Course of Turkey RH During the Chronic Phase

A second group of turkeys, independent of those previously mentioned, was obtained from a commercial producer having an RH problem. From this source nine control and eighteen RH turkeys were selected at four weeks on the basis of ECG criteria previously reported.[6] These poults were fed the same commercial starter ration used for the breeding trial flocks to six

* College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota.
** Veterans Administration Hospital, Minneapolis, Minnesota.
*** Hennepin County General Hospital, Minneapolis, Minnesota.

weeks, and a grower ration from the same source to 13 weeks of age. Control and RH turkeys were weighed and ECG monitored at 4, 5, 7, 9 and 13 weeks of age. At the end of this experiment (13 weeks) all surviving turkeys were sacrificed and autopsied. Based upon ECG and autopsy results, the turkeys were grouped into control, RH-recovered, RH-live and RH-dead groups. The RH-dead group is composed of those poults dying prior to the termination of the experiment, and having ECG records and gross lesions of RH on autopsy. The probability values in Tables IV and V were calculated by Student's "t" and two-tailed distribution.[8] All ECG and phonocardiographic recordings were made with a Sanborn 550 recorder.

RESULTS

Breeding Trials

Flock 1 had higher fertility and hatchability, as well as lower poult mortality and incidence of RH (Table I). In all groups the greatest number of poult deaths occurred during the first two weeks after hatching. Incidence of RH was greater in both pen mated and pedigreed turkeys from Flock 2. In all groups the RH male/female ratio was greater than one.

Incidence of RH in progeny from the pedigreed hens of Flock 2 ranged from 22% to zero (Table II). RH progeny from two hens showed ventricular septal defects (VSD). Both hens were mated to Tom 112. Percentage of progeny with RH from each pedigreed tom ranged from 19% to zero (Table III).

TABLE I.—*Performance of Experimental Breeding Flocks—1971*

	Flock 1 Pen mated	Flock 2 Pen mated	Flock 2 Pedigreed
Infertility (percent)	10.6	23.6	22.8
Hatchability (percent)	81.5	82.7	70.0
Mortality through 35 days of age (percent)	22.1	52.3	79.2
Percent of total 35 day mortality during first 14 days of life	70.0	86.8	90.0
Total poults hatched	275	394	249
Round Heart (RH)	4	40*	18**
RH male/female ratio	4/0	26/14	12/6
Percent RH	0.46	10.2	7.2

* Does not include 3 RH with VSD
** Does not include 2 RH with VSD

TABLE II.—*Incidence of Round Heart in Poults from Flock 2, Pedigreed: Hen Performance—1971*

Hen No.	Total poults	Total RH	Tom RH	Hen RH	Percent RH*
127	12	0	—	—	0
128	10	0	—	—	0
129	18	1	—	—	6
130	5	0	—	—	0
144	12	0	—	—	0
154	36	1	1	—	3
156	1	0	—	—	0
157	23	1	1	—	4
158	10	2	2	—	20
159	0	0	—	—	0
161	15	2**	2**	—	13
162	13	1	—	1	8
163	4	0	—	—	0
164	32	7**	5**	2	22
167	25	0	—	—	0
168	33	5	3	2	15
Total	249	18***	12***	6	7.2%***

* To the nearest percent
** One each VSD
*** Not including VSD cases

Clinical Course of Turkey RH During the Chronic Phase

Identifying ECG features of RH, notably the higher voltage R_X and R_Y, are shown in Figure 2 as contrasted with the control ECG (Figure 1). The phonocardiogram (PCG) of this RH turkey (Figure 2) shows a pansystolic murmur, and a third heart sound (S_{3-4}) which may be a summation of S_3 and S_4.

At four weeks, the control group has low X lead QRS voltages, while the Y lead voltages of −7 reflect the QS or S wave dominance of this lead's QRS complex (Table IV). All RH groups

TABLE III.—*Incidence of Round Heart in Poults from Flock 2, Pedigreed: Tom Performance—1971*

Tom	Total Poults	Total RH	Tom RH	Hen RH	% RH*	Hens Mated to Tom
112	57	11**	9**	2	19	158, 161, 164
126	37	5	3	2	14	159, 163, 168
117	37	2	1	1	5	156, 157, 162
52	30	1	0	1	3	127, 129
118	73	1	1	0	1	144, 154, 167
171	15	0	0	0	0	128, 130
Total	249	18***	12***	6	7.2%***	

* To the nearest percent
** Two with VSD
*** Not including VSD cases

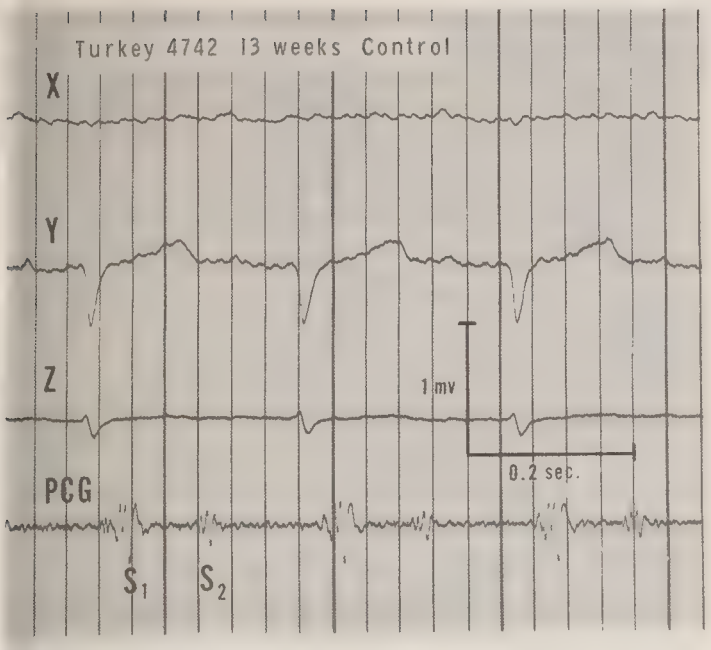


FIGURE 1.—Semi-orthogonal lead system electrocardiographic (ECG) recording of a control turkey at 13 weeks of age. Phonocardiogram (PCG) recorded with microphone placed over left rib area at the site of maximal intensity. Paper speed 200 mm/second.

at this age have high voltage R_X and R_Y waves which result in frontal plane QRS axis deviation and significantly larger mean QRS vector strengths. By thirteen weeks the control pattern shows a slight increase in voltages, while the RH groups deviate.

Some RH turkeys recover spontaneously, evidenced by a transition in the ECG from RH to control patterns by 13 weeks of age (Figure 3). The RH-recovered group is distinguished by reduction in the QRS_X voltage and conversion of the QRS_Y form to a negative value (Table IV). At this age they have a significantly lower mean QRS vector strength and a mean frontal plane QRS axis approaching that of the controls. Five of the eight turkeys in this group made the ECG transition by the ninth week of age. Several in the recovered group had no measurable R_Y wave at 13 weeks.

The RH-live group are RH survivors with essentially the same ECG pattern seen at four weeks, but with slightly lower voltages. Their mean QRS vector strength is not significantly different from that of the controls of the same age. All turkeys of the RH-dead group had RH ECG patterns prior to death.

At four weeks all RH groups had significantly lower mean body weights than the controls (Table V). However, by 13 weeks, both RH-recovered and RH-live groups had body weights not significantly different from the controls.

DISCUSSION

Pen mating experience with our experimental Flock 2 has yielded the following consecutive yearly incidences of RH: 26, 6 and 10.2%. Comparative RH incidences for Flock 1 are: 5, 1.3 and 0.46%. While the incidence of RH in Flock 1 may be higher than the general population, it does serve as a control to which Flock 2 may be compared. Because both flocks have been closed to genetic outcrossing, the consistently higher RH incidence in Flock 2 suggests inheritability of the disease. The high incidence of RH in the progeny from some matings, especially hens mated to Tom 112, suggests a familial mode of inheritance (Tables II and III). While the data are not conclusive, it appears that the tom may

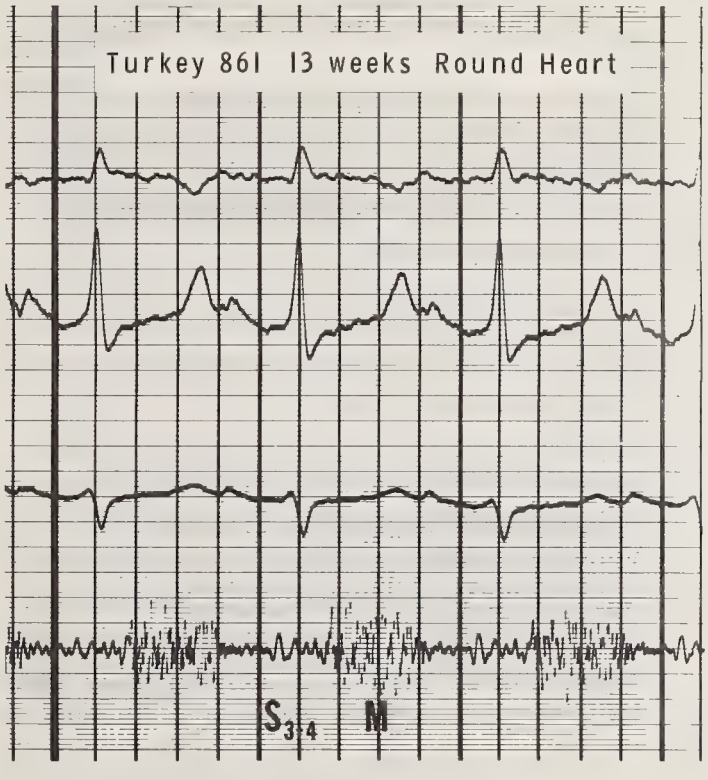


FIGURE 2.—Electrocardiogram and phonocardiogram of round heart diseased turkey. S_{3-4} summation third heart sound. Systolic murmer (M). Same calibration as Figure 1.

TABLE IV.—*QRS Amplitude Data From Electrocardiograms of Control and Round Heart (RH) Groups of Turkeys at Four and Thirteen Weeks of Age**

Age	Parameter	Control	RH recovered	RH Live	RH dead
4 Weeks	Number	9	8	5	5
	X Lead**	1 (4 to −2)	10 (13 to 7)	12 (14 to 9)	5 (14 to −2)
	Y Lead**	−7 (−2 to −4)	5 (12 to −1)	16 (22 to 5)	13 (26 to 3)
	Z Lead**	−1 (0 to −4)	1 (7 to −3)	0 (5 to −5)	−1 (5 to −9)
	Mean vector strength***	7.5 ± 2.8	12.1 ± 2.9	20.4 ± 5.5	15.7 ± 8.8
	Probability	—	<0.01	<0.01	<0.05
13 Weeks	Number	9	8	5	0
	X Lead**	−1 (2 to −3)	4 (7 to 2)	8 (10 to 4)	—
	Y Lead**	−9 (−5 to −12)	−2 (3 to −5)	11 (18 to 2)	—
	Z Lead**	−3 (0 to −7)	−4 (0 to −6)	−4 (−3 to −5)	—
	Mean vector strength***	9.6 ± 3.0	6.6 ± 1.47	14.6 ± 6.0	—
	Probability	—	<0.05	>0.05	—

* All values in mm deflection. To convert to millivolts divide by 30.
** Data are expressed as groups mean, numbers in parenthesis are ranges.
*** Data are expressed as groups mean ± 1 S.D.

be more responsible than the hen for the transmission of, or the susceptibility to, the disease.

Pedigreeing and sex distribution experiences provide an interesting comparison to idiopathic cardiomegaly (IC) and EFE in man. Greater incidence of RH in the male turkey is consistent with IC data reported in Edington's and Jackson's Groups II and III,[9] and in 17 cases reported by Antia.[10] A sharp contrast is noted, however, in the sex incidence of EFE reported by Moller et al.,[11] where the male/female sex ratio was 0.63:1. Turkey RH male/female sex ratios of approximately 2:1 have been consistent, not only in our experimental flocks, but in RH deaths occurring in commercial operations as well. When these commercial flocks are followed to the processing plant (at 12 to 26 weeks of age), those birds with lesions of RH again have a sex ratio of 2:1. One interpretation of these observations is that hens may be better able to recover from the acute phase than toms.

The incidence of RH, as shown in Tables I–III, is based upon autopsy and/or ECG findings. These changes are not commonly manifest before 10–14 days of age. It must be noted that the percent incidence of RH in Table I is based upon all poults hatched. Calculating the incidence of RH on only those turkeys surviving the acute phase would, for example, increase the incidence for the pen mated Flock 2 turkeys about

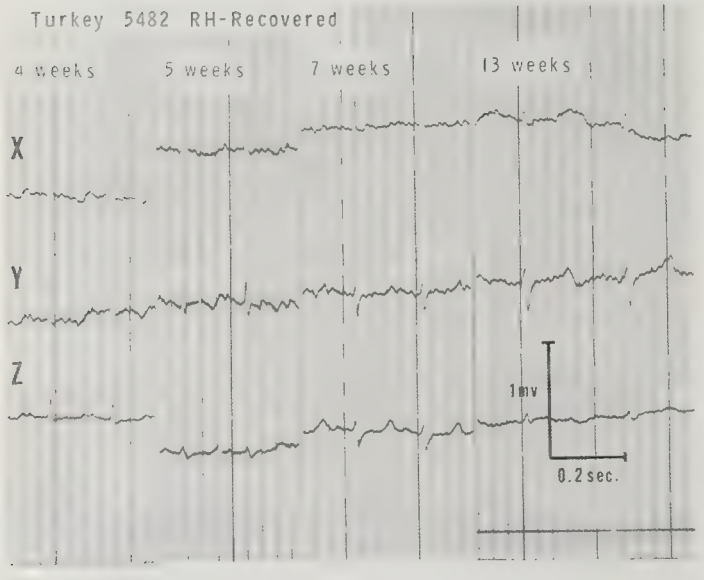


FIGURE 3.—Semi-orthogonal lead system ECG recording of a turkey spontaneously "recovering" from round heart disease. Paper speed 100 mm/sec. The voltages used to compute the mean values in Tables 4 and 5 for this subject would be: Four weeks: X (12), Y (10) and Z (4); Thirteen weeks: X (4), Y (−3) and Z (0).

TABLE V.—*Live Body Weights (kg) of Control and Round Heart (RH) Groups of Turkeys at Four and Thirteen Weeks of Age*

Age	Statistic	Control	RH recovered	RH live	RH dead
4 wks					
	Number	9	8	5	5
	Mean	0.69	0.49	0.45	0.51
		±0.13	±0.05	±0.04	±0.06
	Probability	—	<0.01	<0.01	<0.02
13 wks					
	Number	9	8	5	0
	Mean	5.34	4.72	4.67	—
		±1.34	±0.86	±0.57	—
	Probability	—	>0.05	>0.05	—

Data are expressed as groups mean ±1 S.D.

two times, or from 10.2 to 20%, based on an acute phase mortality of 45% (86.8% of 52.3, Table I).

The initial occurrence of VSD in the 1971 breeding program adds a complicating factor. However, it would appear that VSD is an incidental finding, and that the RH and EFE developing in the five cases from Flock 2 were primary lesions and not a result of the VSD. VSD lesions observed in these 5 cases were similar to those reported by Einzig et al.[12]

The RH turkey can be readily identified at four weeks of age by QRS frontal axis shift and increased QRS vector strength. The gradual return of these parameters toward the control values suggests that many "recoveries" occur spontaneously. This observation has also been made by Hunsaker et al.[7]

Occasionally a turkey with a typical RH ECG at three weeks will convert to control ECG values, and have a grossly normal heart on autopsy at five weeks. The reverse has also been noted, that is: a control ECG pattern at two and three weeks of age, and an RH ECG pattern and lesions at 5 weeks. Evidently a variation in the time course of the disease exists with respect to onset of, and rate of recovery from, the chronic phase. Curiously, the same ECG RH identifying features are present whether the heart, on autopsy, has predominantly right, left or biventricular dilatation. This suggests that the ECG patterns currently used to identify RH turkeys may be primarily related to the pathophysiology of the conduction tissues and not to the associated myocarditis and ventricular dilatation and/or hypertrophy *per se*. These ECG changes of turkey RH are more striking than the ST-T wave changes and left ventricular hypertrophy patterns reported in IC and EFE in man.[11,13]

Einzig et al.,[14] comparing the same groupings of control and RH turkeys at 26 weeks of age, reported that the cardiac outputs of control, RH-recovered and RH-live groups were not significantly different. Both the RH-recovered and RH-live groups had increased cardiac filling pressures and lower systemic arterial pressure. The association of arterial hypotension and low mean QRS vector strength of RH-recovered turkeys is consistent with data reported in a strain of hypotensive turkeys by Krista et al.[15] The degree of involvement of RH in this strain was not reported.

Auscultation over the left rib area of a number of RH turkeys yields a pansystolic murmur as in Figure 2. The conclusion that the murmur is mitral in origin is based upon the following: (1) maximal intensity of the murmur is located over the left rib area, (2) the association of mitral valve pathology with the disease,[4] (3) cineradiographic evidence of mitral insufficiency (MI),[16] and (4) a reported case of RH with a cardiac output curve consistent with MI.[14] The prominent third heart sound (Figure 2) is consistent with a diagnosis of congestive heart failure associated with MI. The incidence of murmurs in RH turkeys in this study is unknown. However, 6 of 20 (30%) in another group of RH tom turkeys at five weeks of age had pansystolic murmurs as presented in Figure 2. Whether the mitral valve pathology is subsequent to the left ventricular dilatation, or results from primary valvulitis is unknown. It is interesting that only the mitral valve has been observed to be involved in turkey RH. The association of MI and cardiomyopathy in man is also recognized by investigators.[11,17]

Histological examination of hearts from the RH-recovered group shows lesions that are intermediate between the EFE of the RH-live or RH-dead groups and the control turkeys. Evidence of EFE of the left ventricle persists, and a degree of left ventricular dilatation and hypertrophy were observed at the time of sacrifice (13 weeks). Morphological residual changes probably persist in all but the most mildly involved cases.

Many of our turkey cases may be classified as IC and would fit Goodwin's[18] classification of primary congestive cardiomyopathy. The autopsy findings of a wide spectrum of ventricular dilatation and EFE in turkey RH suggest that the presence of EFE may be secondary to the myocarditis. The hypothesis of Black-Schaffer[19] that EFE is the result of dilatation of a high pressure pulsatile chamber appears to be valid for turkey RH.

The presence of myocarditis and virus-like particles in the embryos and turkeys from this experimental flock, as previously reported,[4,20] is consistent with *in ovo* transmission of the

disease and a familial mode of inheritance.

The authors[21,22] have demonstrated a reduction in death losses of poults during the acute phase of the disease with immunosuppressive levels of cortisone. The improved survival rate of susceptible turkeys was not accompanied by any morphological changes upon examination by both light and electron microscopy. It was postulated that the mechanism of injury, if it is related to the virus-like particles, may not be due to their presence in the cell, but to some immunologic process. The suggestion was made that cortisone may influence the immune response more than the inflammatory reaction.

SUMMARY

Turkey Round Heart Disease appears to have a familial mode of inheritance.

The RH male/female sex death ratio during the chronic phase is approximately 2:1.

Fifty percent of the RH turkeys with RH at four weeks of age will spontaneously "recover" by 13 weeks of age.

Approximately 30% of four to five week old RH turkeys have cardiac murmurs consistent with mitral insufficiency.

Turkey round heart disease is an appropriate model for the study of cardiomyopathy.

ACKNOWLEDGMENTS

We want to thank Mr. Walter Hunsaker, Canadian Department of Agriculture, Ottawa, Ontario, Canada, for contributing hatching eggs from which Flock 2 was originated, and Dr. B. S. Pomeroy, University of Minnesota, for donating turkeys for Flock 1 foundation stock. We also wish to acknowledge the excellent turkey care provided by Mr. Carl W. Edborg, III, and the able assistance of Mr. Virgil Voigt, Mr. Bruce Hultgren, and Mr. James Reiser.

This research was supported in part by the Minnesota Turkey Growers' Association, the Dwan Family Fund, and the Minnesota Heart Association.

REFERENCES

1. Magwood, S. E., and Bray, D. F. Disease condition of turkey poults characterized by enlarged and rounded hearts. Canadian J. Comp. Med. 26:268–272, 1962.
2. Sautter, J. H., Newman, J. A., Kleven, S. H., and Larsen, C. T. Pathogenesis of round heart syndrome in turkeys. Avian Diseases 12:614–628, 1968.
3. Jankus, E. F., and Good, A. L. Round heart disease in turkeys. Minn. Vet. 10:11–12, 1970.
4. Noren, G. R., Staley, N. A., Jankus, E. F., and Stevenson, J. E. Myocarditis in round heart disease of turkeys. Virchow's Archiv. Abteilung A. Pathologische Anatomie 352:285–295, 1971.
5. Hunsaker, W. G. Round heart disease in four commercial strains of turkeys. Poultry Sci. 50:1720–1724, 1971.
6. Jankus, E. F., Good, A. L., Jordan, K. A., and Saxena, S. K. Electrocardiographic study of round heart disease in turkey poults. Poultry Sci. 50:481–486, 1971.
7. Hunsaker, W. G., Robertson, A., and Magwood, S. E. The effect of round heart disease on the electrocardiogram and heart weight of turkey poults. Poultry Sci. 50:1712–1720, 1971.
8. Steel, R. G. D., and Torrie, J. H. *Principles and Procedures of Statistics*, McGraw-Hill, New York, pp. 67–72, 1960.
9. Edington, G. M., and Jackson, J. G. The pathology of heart muscle disease and endomyocardial fibrosis in Nigeria. J. Path. and Bact. 86:333–344, 1963.
10. Antia, A. U. Idiopathic cardiomegaly in african children. Pathologia et Microbiologia 35:44–48, 1970.
11. Moller, J. H., Lucas, R. V., Adams, P., Anderson, R. C., Jorgens, J., and Edwards, J. E. Endocardial fibroelastosis; a clinical and anatomic study of 47 patients with emphasis on its relationship to mitral insufficiency. Circulation 30:759–782, 1964.
12. Einzig, S., Jankus, E. F., and Moller, J. H. Ventricular septal defect in turkeys. Am. J. of Vet. Res. 33:563–566, 1972.
13. Sekiguchi, M., and Konno, S. Diagnosis and classification of primary myocardial disease with the aid of endomyocardial biopsy. Jap. Cir. J. 35:737–754, 1971.
14. Einzig, S., Jankus, E. F., and Moller, J. H. Round heart disease in turkeys: A hemodynamic study. Am. J. Vet. Res. 33:557–561, 1972.
15. Krista, L. M., Jankus, E. F., Waibel, P. E., Sautter, J. H., Shoffner, R. N., and Quarfoth, G. J. Comparison of electrocardiograms of hypertensive and hypotensive male turkeys. Poultry Sci. 49:700–703, 1970.
16. Jankus, E. F. Unreported data.
17. Rodin, A. E., Harris, L. C., and Nghiem, Q. X. Pediatric cardiomyopathies. J. Mol. and Cell. Card. 3:63–79, 1971.
18. Goodwin, J. F. Congestive and hypertrophic cardiomyopathies. The Lancet, 731–739, April 11, 1970.

19. BLACK-SCHAFFER, B. Infantile endocardial fibroelastosis. A. M. A. Arch. Path. 63:281–306, 1957.
20. STALEY, N. A., NOREN, G. R., and JANKUS, E. F. Myocarditis in turkeys: association with virus-like particles. Am. J. Vet. Res. 33:859–861, 1972.
21. NOREN, G. R., STALEY, N. A., and JANKUS, E. F. Effect of cortisone acetate upon the early mortality of naturally occurring interstitial myocarditis in turkeys. (Abstract) Circulation 44 (Sup. No. 2):107, 1971.
22. STALEY, N. A., NOREN, G. R., and JANKUS, E. F. Early response of a naturally occurring cardiomyopathy to corticosteroids. Presented at the American Association of Pathologists and Bacteriologists Meeting, Cincinnati, Ohio, March 11–18, 1972.

DISCUSSION

CHAIRMAN KEZDI: In clinical medicine one of the most frustrating problems is idiopathic cardiomyopathy. Presently we can do very little for these patients. It is most interesting that the same problems exist in animals and a good model for the above probably is available. Maybe we can learn something about the immunopathology related to this problem.

M. R. MALINOW, Beaverton, Oregon: Are you able to transmit this disease to other animals and do you find lesions in some other organs? You mentioned avian lymphomatosis. Do you also find lesions in the coronary arteries similar to those which can be found in chickens with lymphomatosis?

DR. JANKUS: With regard to your first question, we attempted a couple of years ago to make crude filtrates of infected hearts and inoculate them into male one-day old poults or newly hatched poults. We were unsuccessful in reproducing the disease. However, we sacrificed these poults at about four weeks of age and did not subject them to histological examination which was an oversight on our part. But they did not develop gross lesions.

DR. MALINOW: My second question was: do you find lesions in some other places besides the myocardium?

DR. JANKUS: Lesions are characteristically located in the left heart. EFE has been observed only in the left ventricular chamber.

DR. MALINOW: I am asking whether lesions are present in other organs, like the kidney or the liver, or in the central nervous system.

DR. JANKUS: Congestion of the liver and lungs has been observed. Sautter, in 1968, reported bile duct hyperplasia. We have not observed, nor are there any reports, to my knowledge, of significant lesions in the renal, pulmonary or central nervous systems.

DR. MALINOW: I believe that the last question was: did you find any lesions in the coronary arteries?

DR. JANKUS: We have observed no lesions in the coronary arteries. Occasionally, perivascular cuffing of coronary arteries by mononuclear cells is observed.

DR. C. GRANT LA FARGE, Children's Hospital Medical Center, Boston, Mass.: You quote my old friend John Goodwin and Celie Oakleaf's work in cardiomyopathy. Do you ever see any obstructive myopathy in these birds?

DR. JANKUS: No. Certainly not of the subaortic stenosis type. Relative to congenital heart disease we have observed VSD lesions in a few birds. In every VSD case we also have observed EFE. It is our opinion at this time that the EFE was "primary" and the VSD was coincidental. All of the VSD cases have been of the membranous septum type about a millimeter or two in diameter. These observations will be published shortly in the American Journal of Veterinary Research; Einzig, Jankus and Moller.

DR. W. ERLICH, Johns-Hopkins University School of Medicine, Baltimore, Md.: (Asks whether evidence indicates the presence of a virus.)

DR. JANKUS: That's a good question. Our reply is: Yes, probably. Of course, until you can satisfy Koch's postulates you cannot prove that this is a virus disease. The consistent finding of the virus in the diseased tissue is the best information available to us together with the disease process itself. But we have no convincing evidence by any means.

CHAIRMAN: This is a tremendous clinical problem as well: to determine which is a virus disease and which isn't.

DR. LA FARGE: Have you tried therapeutic regimens, other than the cortisone regimen in the poults?

DR. JANKUS: No. In field outbreaks our advice has been general supportive therapy such as

instituting a low salt diet, removing all feed additives and decreasing light intensity to restrict activity.

CHAIRMAN: I have a question. Did any of these animals recover completely?

DR. JANKUS: That is difficult to answer. As we reported, about 50 percent of poults found to be diseased at four weeks of age make an apparent recovery. Grossly and by light microscopy some residual dilatation and endocardial fibrosis persist. We believe there is an entire spectrum of the "recovery" state, and the younger the bird is when "recovery" is initiated, the less the residual lesions. Perhaps all progeny are diseased when hatched and those that make a complete recovery we call normal. I believe spontaneous recovery has also been reported in human cases of idiopathic cardiomegaly.

STUDIES OF A CARDIOGENIC SHOCK MODEL

P. Kezdi, S. N. Misra, R. K. Kordenat and T. J. Smith*

Selective embolization of the circumflex branch of the left coronary artery in the dog with minute amounts of metallic mercury resulted in predictable cardiogenic shock and pump failure and enabled us to study the natural history, the effect of drug interventions, and calculations of myocardial energetics in this condition. Decrease of left ventricular dP/dt and cardiac output were the two most reliable hemodynamic parameters in predicting irreversible shock and early death. Continuous decline of these parameters below 50% of control after the first hour of shock was a bad prognostic sign. These findings corresponded with clinical observations of others that the fundamental pathophysiologic change in shock is depressed myocardial contractility. In addition, end-diastolic and end-systolic volumes, determined by dye dilution technique, showed significant increase along with a significant decrease in ejection fraction during the shock state. The calculation of energetics of the heart, including wall force and tension along with fiber shortening and contractile element work, corresponded with the above findings. In a group of experiments, the effect of drug interventions was studied when hemodynamic deterioration reached the irreversible shock state. Alpha and beta adrenergic blockade and stimulation alone and in combination were studied. Significant hemodynamic improvement, along with improvement of kidney and other vital functions, was observed with combined use of carefully titrated minute doses of norepinephrine and isoproterenol. The dose range tested was that commonly used clinically. The value of combined use of the above drug treatment in human myocardial infarction with shock was confirmed in five patients who showed marked improvement. This is a predictable and reproducible experimental model of shock and pump failure which provided further insight into underlying physiological derangements and helped to delineate the best pharmacologic intervention. The experience in the experimental laboratory could be directly extrapolated to the clinical state.

INTRODUCTION**

The great increase of myocardial infarction generated much interest for the development of animal models which simulate human myocardial infarction. Models have been designed for the study of hemodynamic mechanisms during the development and course of myocardial infarction and for the study of its complications such as arrhythmias and cardiogenic shock. We have been interested in suitable models of myocardial infarction resulting from occlusion of a large coronary artery and of myocardial infarction with cardiogenic shock by some other technique since shock usually does not develop with the acute occlusion of a large coronary artery in animals. A model of myocardial infarction with shock will closely simulate the human condition only if:

1. It can be produced in a closed chest animal;
2. Observations can be made in the unanesthetized, unrestrained state; and
3. Natural history and mortality can continually be observed.

The multiple techniques proposed usually do not fulfill all of these criteria.[1-3] It appeared to us that the best suited technique for this purpose is the one originally reported by Lluch et al.[4] who used selective injection of metallic mercury into the coronary arteries. While this technique does not result in narrowing or obstruction of a major coronary artery, its end result on the affected myocardium is very similar in that it leads to localized hypoxia, functional impairment and necrosis of the myocardium, and a clinical picture very similar to human cardiogenic shock. We have therefore adapted this technique for our studies and slightly modified it, predominantly by applying a special catheter developed in our laboratory, which allows selective embolization of any of the branches or the main branch of the circumflex coronary artery. This report describes the technique, the hemodynamic results and the various

* Cox Heart Institute, Kettering Medical Center, Dayton, Ohio.

** This work was supported by NIH Grant HE-09885 and in part by Contract PH-43-68-688.

studies we have undertaken by utilizing this shock model.

TECHNIQUE

In order that control studies may be performed in the awake sedated animals, a double catheter was designed with a smaller catheter inside a larger one.[5] The smaller inner catheter can be left in the circumflex coronary artery without interfering with coronary blood flow until the time of injection of the mercury. This catheter has been described previously by us and was also used for the wire technique to produce localized thrombotic occlusion of a large coronary artery which will be described in detail in this meeting by Dr. Stanley. The large outer catheter is preformed with two curves: the one about 9 mm from the tip, the other a mild curve 25 mm from the tip giving an overall 45° curve of the entire catheter (Figure 1). The catheter leans with its second curve, against the wall of the aorta, while the tip is directed toward the left main coronary artery. The small inner catheter, which is a PE 90 polyethylene catheter, is then advanced through the outer catheter into the left coronary artery and directed to the circumflex branch. The position is verified under image intensifier with small amounts of dye injections. (Figure 2 a and b) The inner catheter can then be left in place until the time of mercury injection without interfering with the coronary blood flow. The outer catheter is slightly withdrawn to avoid blocking of the ostium of the left coronary artery.

Initially the animals are anesthetized with intravenous sodium thyopenthal (10 mg/kg) and cutdowns are made using xylocaine to infiltrate the skin. The coronary catheter is introduced

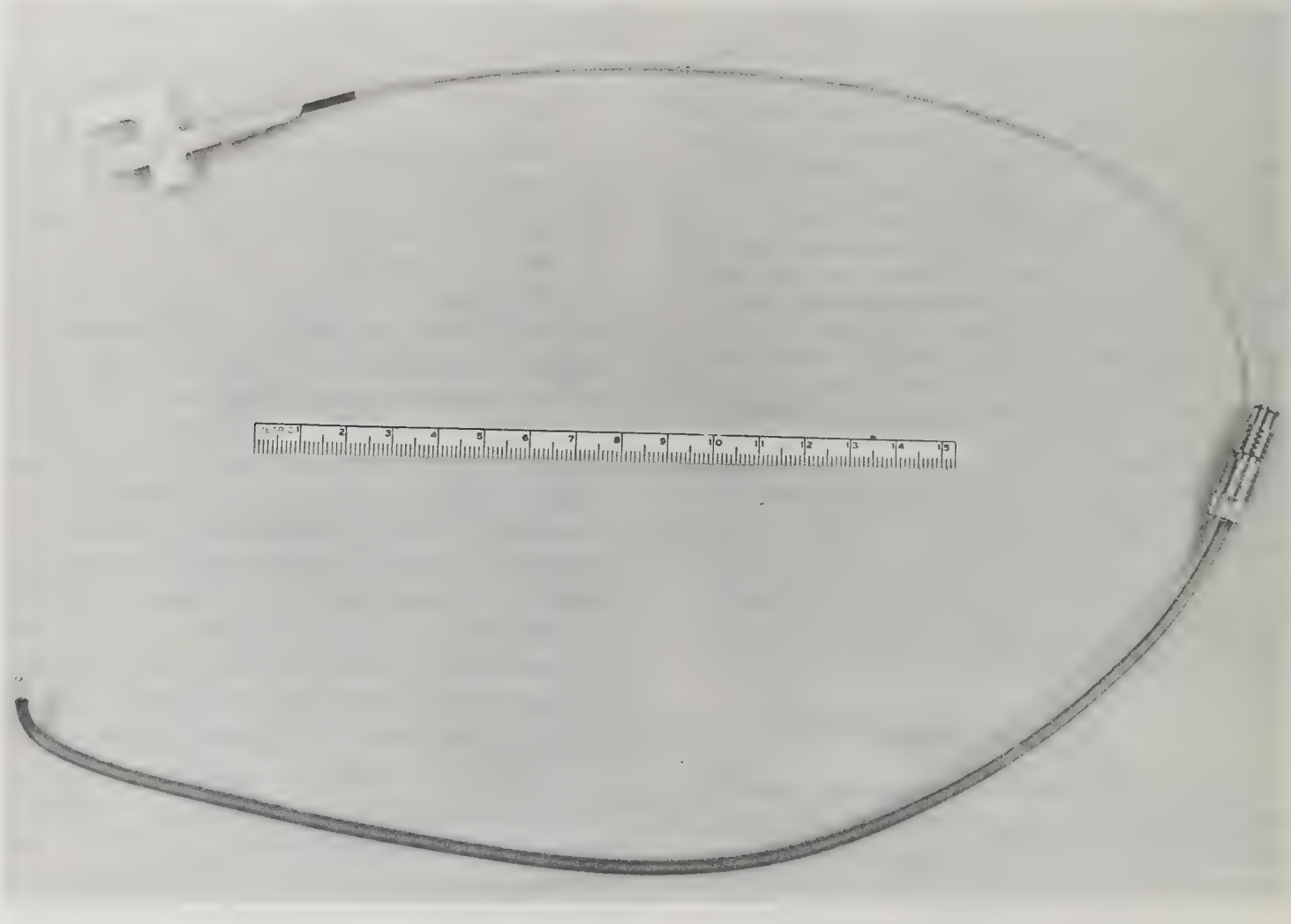


FIGURE 1.—Double catheter assembly for selective catheterization of coronary arteries in dogs.



FIGURE 2.—a. Selective visualization of circumflex coronary artery in a dog (arrow). b. The inner catheter is placed in the circumflex coronary artery (arrow).

through the right common carotid artery which is exposed together with the external jugular vein. The catheter, placed through the jugular vein, is advanced to the right atrium, the pulmonary artery, or the coronary sinus depending upon the experimental protocol. A second arterial catheter is placed through the femoral artery and advanced into the left ventricle. The surgical exposures are then sutured and the wounds bandaged. At the end of the procedure the animal is usually awake. The animals are then given morphine sulfate, 15 mg IM, approximately in hourly intervals to maintain slight sedation. They are placed on their side on a blanket and covered to maintain body warmth. Water is given to them *ad libitum*.

The left ventricular and arterial catheters, the pulmonary artery catheters are connected to pressure gauges and pressures are continuously monitored. A single lead electrocardiogram is also monitored. Cardiac outputs are performed in duplicate by the dye dilution technique using 2.5 mg indocyanine green as a bolus in the pulmonary artery. Arterial blood is withdrawn through a Gilford densitometer using a Harvard constant withdrawal infusion pump. Arterial, venous, and coronary sinus blood samples can be withdrawn and O_2, CO_2 tension, pH, lactates, pyruvates, electrolytes, and serum enzymes be determined. In a number of experiments, we have determined myocardial oxygen consumption and lactate production and calculated excess lactate according to the formula of Huckabee. Blood volumes were also measured by the radioactive I^{131} tagged albumin technique. In another series of experiments, left ventricular end diastolic and end systolic volumes and cardiac output were obtained in duplicate by the method of Shaffer.[6] Ejection fraction was calculated from this data.

Following the baseline studies to determine the hemodynamic and biochemical variables, .2 ml metallic mercury is injected into the catheter previously placed in the circumflex artery and

this is flushed by 2 ml isotonic saline. The inner catheter is then withdrawn and removed while the outer catheter is left in place.

Soon after the injection of mercury electrocardiographic changes of injury pattern occur and all hemodynamic measurements deteriorate. The animals usually do not show any sign of pain or only occasionally become somewhat restless. In such case, additional morphine is given. The measurements are then repeated serially for several hours and, depending upon the experimental protocol, are continued during the entire day, until the death of the animal, or until termination of the experiment.

RESULTS

We have used this animal model to study the hemodynamic and biochemical changes in the cardiogenic shock and attempted to determine prognostic factors for early death.

In another series of experiments we have studied the role of vagal afferents originating in the left heart in the mechanism of shock state. It has been known for sometime that reflexes originate in the heart and these reflexes may be both chemoceptor and baroceptor in nature. Since these experiments involved recording of nerve action potentials from the aortic baroceptor, the vagal afferent nerves and postganglionic sympathetic nerves, the animals were kept in general anesthesia for this experiment using chloralose urethan.

In another series of experiments we have tested the effect of sympathetic beta and alpha stimulator and blocking agents in order to determine the best drug or combination treatment of the hemodynamic and metabolic derrangements of cardiogenic shock. The results of these studies are briefly described.

Hemodynamics of Experimental Cardiogenic Shock

In a total of 25 mongrel dogs the hemodynamics and biochemical changes in experimental cardiogenic shock were studied in the awake sedated animal. The purpose was to determine which hemodynamic and biochemical variables describe more closely the derrangements in experimental cardiogenic shock and which can be used to arrive at a prognostic index of early death. Further, we have tested, by subsequent autopsy examinations, the question of how this index relates to the size of anatomical damage of the left ventricular free wall.[7]

Of the 25 dogs thus studied, seven developed ventricular fibrillation within a few minutes after injection of mercury and could not be resuscitated. The remaining 18 dogs were continuously monitored for the entire day. Seven of them (39%) died 2–12 hours after injection while eleven (61%) survived 2–11 days. Since the circumflex artery of the dog supplies the posterior inferior wall of the left ventricle, the changes in the electrocardiogram were reflected in leads 2, 3, and AVF. These changes were marked ST segment elevation leading eventually to Q wave development and T wave inversion. The mercury droplets blocked the smaller and medium vessels of the circumflex coronary artery and produced an infarction of the posterior-inferior wall of the left ventricle. The average heart weight of the total group was 154.17 $\pm$ 15.7 grams and the average infarct weight 36.44 $\pm$ 8.2 grams. This latter represented 42% of the weight of the left ventricular free wall.

All hemodynamic variables showed a significant impairment consisting of decrease of the mean blood pressure, the cardiac output, left ventricular dP/dt max and an increase of the heart rate and peripheral resistence as well as of the left ventricular end-diastolic pressure. These changes occurred during the first hour of the shock and continued to become worse in the subsequent hours (Figures 3 and 4).

In our attempt to determine the prognostic indices which could predict an early death or survival of the initial insult of infarction, we arbitrarily separated the animals into two groups: one which died within 48 hours and the other which survived beyond 48 hours. We then compared the hemodynamic and biochemical variables in the two groups. It can be seen in Figures 3 and 4 that heart rate increased in both groups simultaneously while mean blood pressure decreased to moderately low levels. There was no statistical significance between the two groups. Left ventricular end-diastolic pressure increased in both, somewhat more in

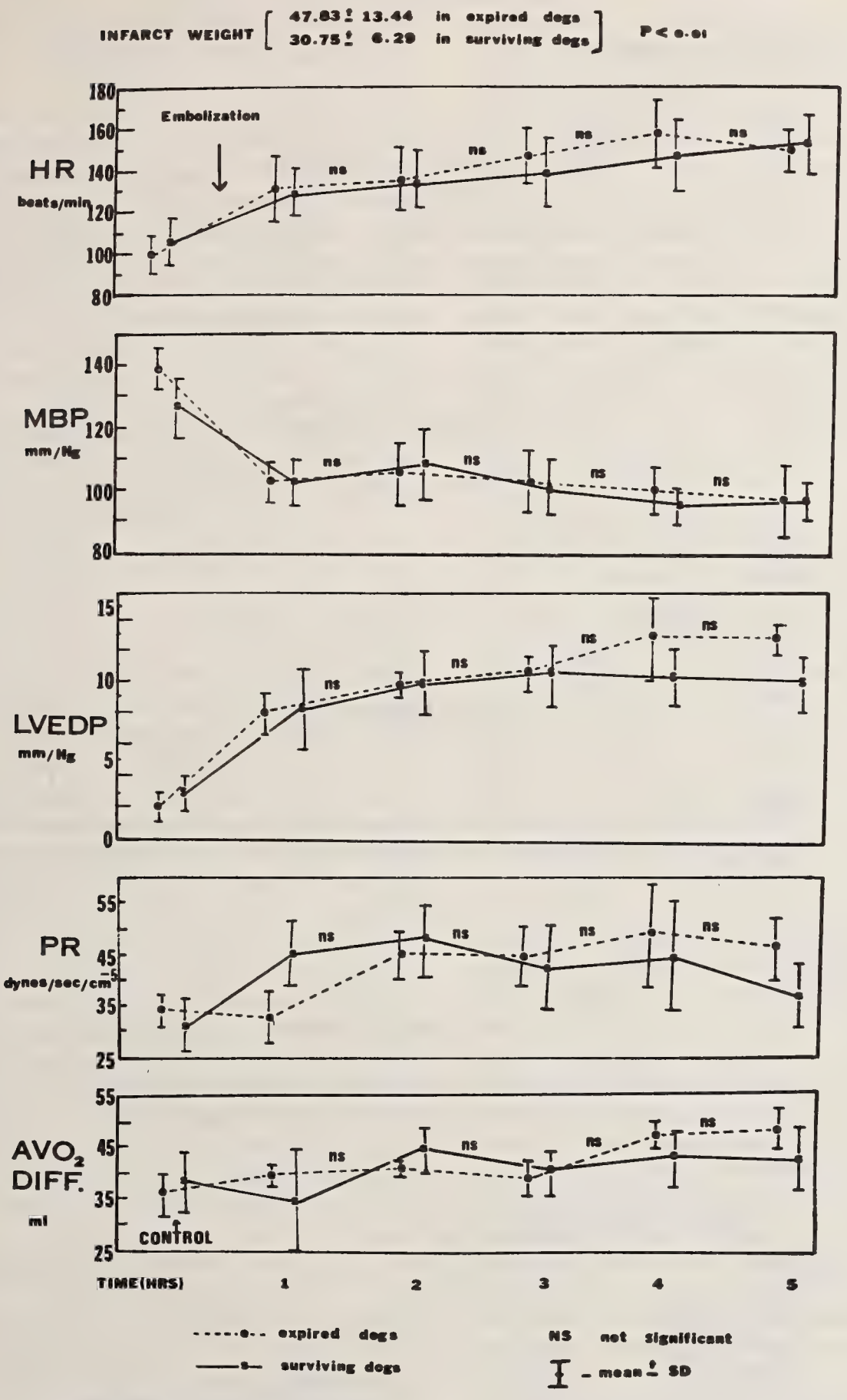


FIGURE 3.—Hemodynamic changes in experimental cardiogenic shock. Comparison of animals surviving longer than 48 hours (solid line) and expired within 48 hours (interrupted line). Vertical bars are standard deviations of means. Time is in hours.

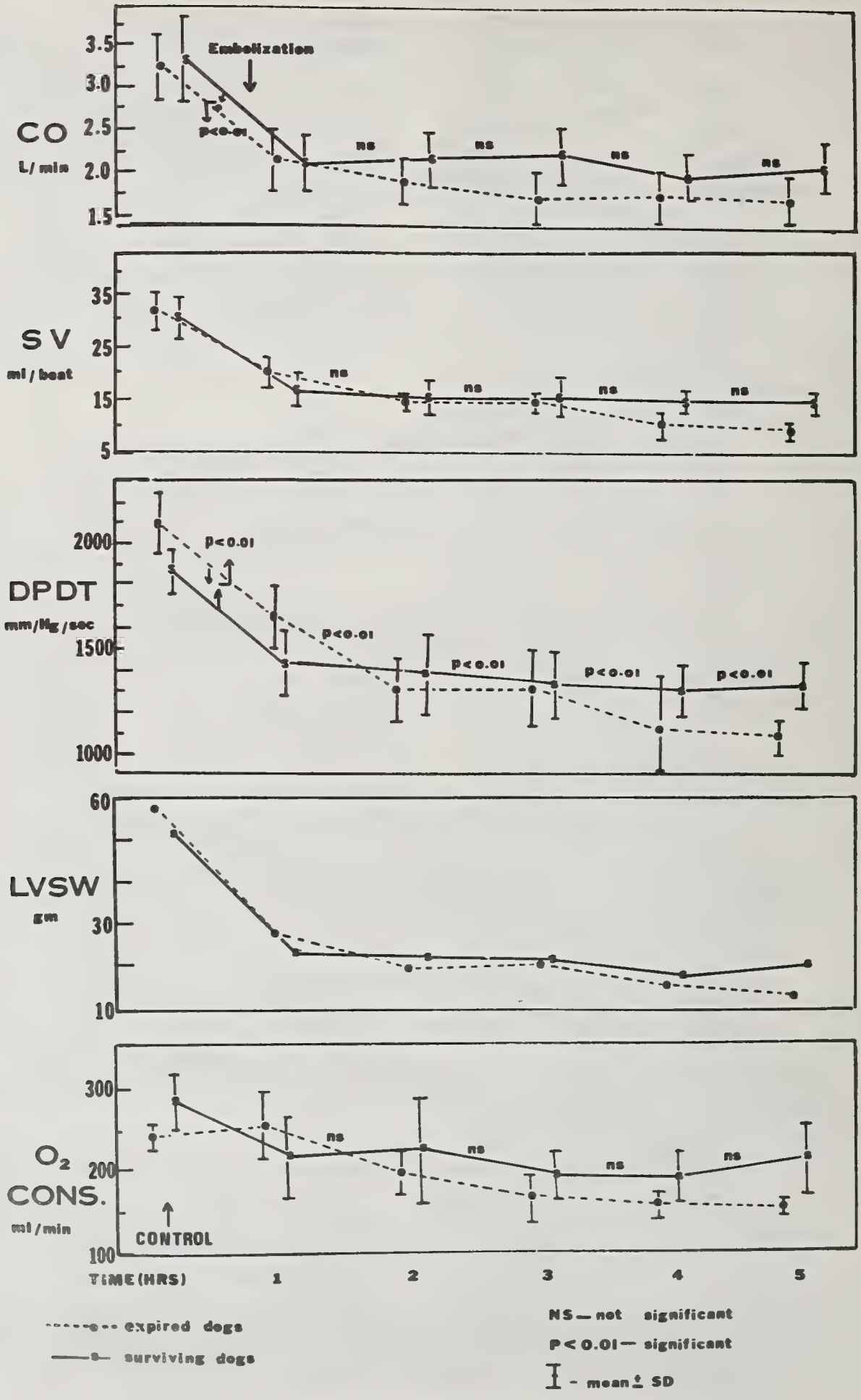


FIGURE 4.—Same as in Figure 3.

the animals with early death but this difference was only of borderline statistical significance. Cardiac output decreased markedly to about 50% of the baseline in both groups, with the nonsurviving slightly lower at the end of five hours. The same was true for the stroke volume. Peripheral resistence increased in both groups. The most significant variable which showed statistically significant difference after five hours of monitoring was the left ventricular dP/dt with the animals surviving longer than 48 hours showing a tendency toward improvement while the ones which died showing a continuous deterioration. This difference in dP/dt was not apparent in the initial phase of monitoring. Oxygen consumption, cardiac output and stroke volume paralleled this difference in dP/dt to a lesser degree.

When the average infarct weight of the early expired and animals surviving more than 48 hours was compared, it was found that the expired dogs had 47.83 $\pm$ 13.44 grams while the survivors 30.75 $\pm$ 6.29 grams of infarct weight. This difference was statistically significant at the 1% level. The total heart weight was not different between the two groups.

Thus, it appeared that initially no hemodynamically significant differences could be detected between the longer surviving and early expiring animals. After five hours of shock the left ventricular dP/dt, the stroke volume, left ventricular end-diastolic pressure, cardiac output and oxygen consumption, in this order, were the most significant prognostic differentiating signs. This difference in hemodynamic variables seems to relate clearly to the percent involvement of left ventricular free wall in the infarction and the early death of these animals was probably due to the greater degree of so called pump failure. These findings closely correspond with the findings of Lluch et al.[4]

Study of Vagal Afferents

It has been long suspected that vagal afferents from the heart may play a role in the regulation of circulation during myocardial infarction and cardiogenic shock. The organism relies in part on circulatory refiex mechanisms compensating for the hypotension, the decrease of stroke volume and cardiac output. The systemic baroceptors play a major role in these reflexes. The question arose whether these compensatory mechanisms are always optimally working in a given shock state. If vagal afferents from the heart are activated during myocardial infarction they may overcome the attempt by the baroceptors to compensate and may, in fact, act as a positive feedback mechanism. In addition to the hemodynamic measurements we have exposed the vagus nerves bilaterally and one aortic baroceptor nerve for nerve impulse recording. Postganglionic sympathetic activity was recorded from a renal nerve. The renal nerve was exposed by a posterior retroperitoneal approach. The technique has been described previously elsewhere.[8]

Following baseline recordings of cardiac output, blood pressures, aortic and sympathetic nerve activity cardiogenic shock was produced by mercury injection through previously placed coronary catheter. The trend of hemodynamics and of the nerve activity was then followed by repeated recordings. The usual hemodynamic changes with 50% decrease of cardiac output, blood pressure decrease and tachycardia developed in these animals. Simultaneously with the decrease of the mean pressure, withdrawal of the aortic nerve activity occurred representing a general withdrawal of systemic baroceptor activity (Figure 5). It was expected that a reflex increase of sympathetic activity should occur. Contrary to this expectation sympathetic activity also decreased, indicating that no maximal vasoconstriction occurred (Figure 6). Thus, the increase in peripheral resistence was probably due to the greater decrease of cardiac output than blood pressure (Figure 7). When, at this time, the vagus nerve was cut or cold blocked, while leaving the aortic and the carotid sinus nerves intact, an increase of sympathetic activity could be demonstrated in most instances concommitently with an increase of systemic blood pressure, heart rate and cardiac output. Aortic nerve activity simultaneously increased as a result of increased mean pressure (Figures 6 and 7).

These findings clearly demonstrated that afferent impulses in the vagus, probably originating in the heart, overcome the baroceptor with-

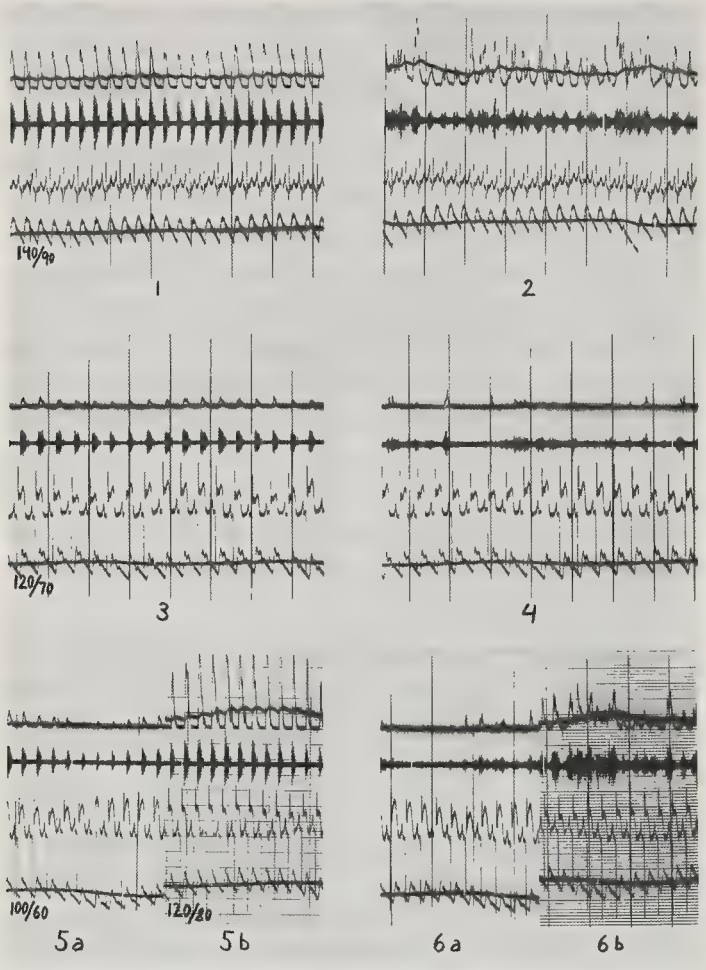


FIGURE 5.—Aortic nerve (left panels) and renal postganglionic sympathetic nerve (right panels) activity in experimental cardiogenic shock. In each panel from top to bottom the recordings represent the following: integrated and mean nerve activity; actual nerve recording; ECG; aortic pressure (with mean pressure). Panels 1 and 2: control recordings; panels 3 and 4: recording during cardiogenic shock state; panels 5a and 6a: recording before cutting vagi (shock state); panels 5b and 6b after cutting vagi. Note decrease of aortic (3 and 5a) and sympathetic (4 and 6a) activity in shock and increase after cutting vagi (5b and 6b).

drawal and inhibit the expected compensatory increase of sympathetic discharge. From other experiments in non-shock animals, we have reason to believe that these reflexes originate in the left ventricle and are activated by increased stretching of the ventricular wall due to the acutely increased end-diastolic volume present in cardiogenic shock. The Bezold-Jarish reflex may also participate initially. We are trying at the present to separate the function of these two types of reflexes in cardiogenic shock.

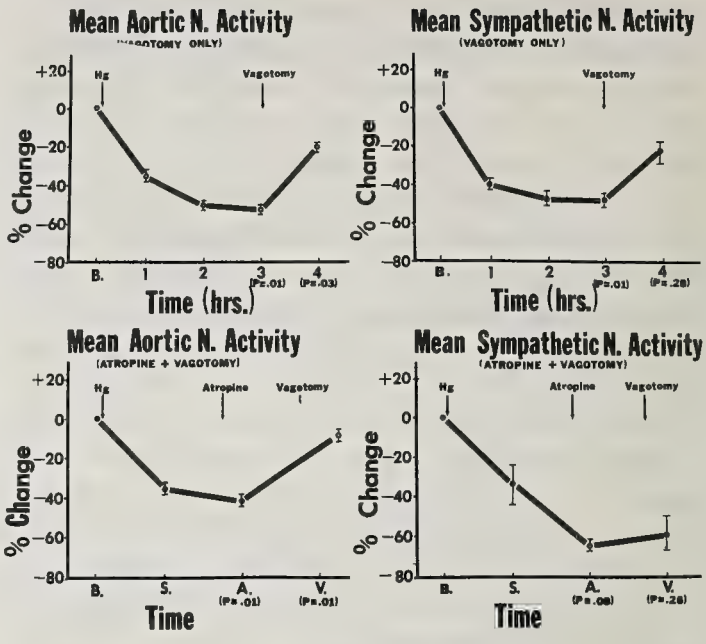


FIGURE 6.—Average aortic nerve and sympathetic nerve activity before and during shock and after vagotomy (upper panels) in eight dogs and the response to atropine and vagotomy during shock in five dogs (lower panels). Vertical lines are standard errors of the mean.

The reported findings of increased catecholamines during myocardial infarction may not be contrary to the decreased sympathetic activity since they originate mainly in the adrenal medulla and not in the sympathetic nerve

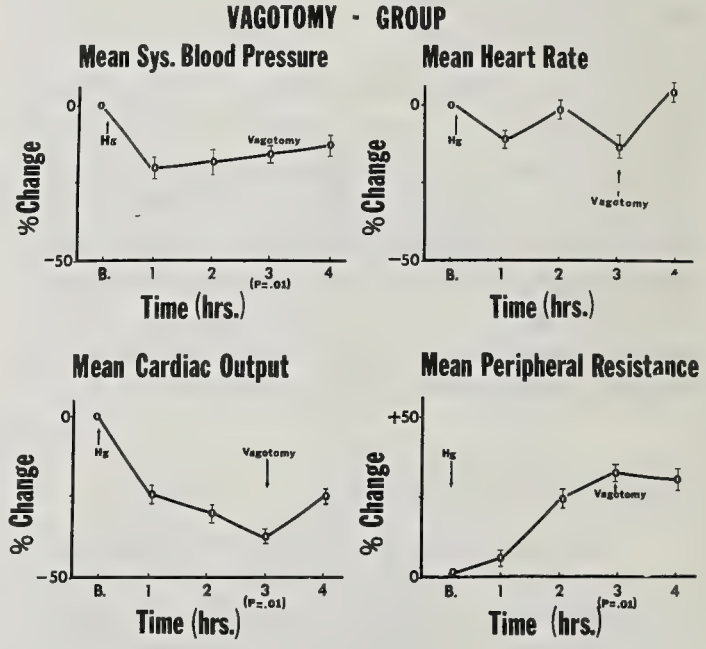


FIGURE 7.—Hemodynamic changes in eight dogs before and after cardiogenic shock and after vagotomy.

endings. It might be worthwhile to mention that atropine did not have the same effect as vagotomy because it only increased heart rate but sympathetic activity and cardiac output remained unchanged (Figure 6). Atropine blocks the efferent outflow of the vagus to the heart and thus acts on heart rate only.

Studies of Sympathetic Augmentation and Block in Cardiogenic Shock

In view of the findings that sympathetic activity is not maximal, but actually depressed in cardiogenic shock (which probably affects both the peripheral resistence vessels and the pumping force of the heart itself), and since atropine does not seem to reverse this trend, we have decided to study systematically sympathetic acting drugs in this cardiogenic shock model. In a total of 50 unanesthetized shock dogs different alpha adrenergic blocking and alpha and beta adrenergic stimulating drugs were tested as to their effect on hemodynamics and 24-hour survival. Table I shows the experimental groups and the drugs tested. Table II shows the direct and the derived measurements obtained. These drugs were administered several hours after the mercury injection when cardiogenic shock has developed with 50% decrease of cardiac output and the other signs of hemodynamic deterioration as previously shown.

Group A received phenoxybenzamine together with volume expansion according to Dietzman and co-workers.[9] Figures 8 and 9 show that the average mean blood pressure further decreased in these animals with the concomitant falling of systemic peripheral resistence. The latter decrease was significant at the 2% level. Cardiac output slightly increased and there was a slight increase of stroke volume from the shock level. However, while the aver-

TABLE I.—*Experimental Cardiogenic Shock Groups of Dogs Studied*

Group	No. of dogs	Drug administered	Dose	24-Hour survival
A	8	Phenoxybenzamine (Dibenzyline)	1–2 mg/kg	1
B_1	8	Norepinephrine	2–4 μg/min	3
B_2	8	Norepinephrine	4–6 μg/min	4
B_3	8	Norepinephrine	6–8 μg/min	5
C	8	Phenoxybenzamine +	– mg/kg	2
		Norepinephrine	8 μg/min	2
D	10	Isoproterenol alone	3–4 μ/min	–
		and		
		Isoproterenol +	1 mg/min	6
		Norepinephrine	2–4 μg/min	6
	50			

TABLE II.—*Parameters Recorded or Calculated*

Measurement	Technique
ECG	
Aortic pressure—systolic—diastolic—mean	Aortic catheter
Cardiac output (CO) Indocyanine green	Lexington C.O. computer and Triangulation
Stroke volume (SV) CO ml/beat	
Left ventricular end-diastolic pressure (LVEDP)	Catheter
Left ventricular dP/dt mmHg/sec	RC differentiating circuit
Peripheral resistence dynes/sec/cm^{-5}	
Left ventricular stroke work (LBSW) g-m	SVx MAP-RAP x 13.6/1000
Endsystolic volume (ESV) ml	Shaffer Indocyanine Green
Enddiastolic volume (EDV) ml	Shaffer Indocyanine Green
Ejection fraction (EF)	SV/EDV
Modified tension time index (PRI) mm Hg per minute	Mean systolic pressure times heart rate
Left ventricular excess lactate moles/lit.	Coronary sinus—arterial lactate minus coronary sinus—arterial pyruvate times arterial lactate/pyruvate
Blood volume	I^{131} tagged albumin

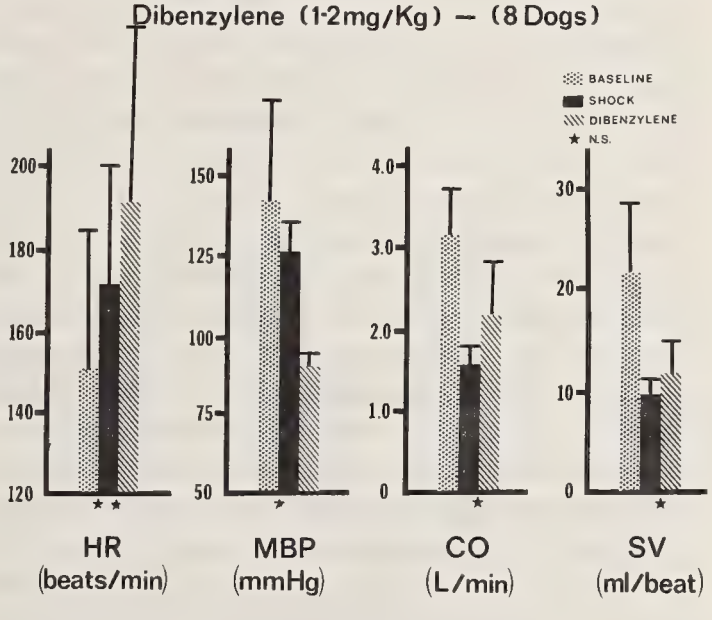


FIGURE 8.—Hemodynamic changes before and during shock and after phenoxybenzamine plus volume expansion. HR = heart rate; MBP = mean blood pressure; CO = cardiac output; SV = stroke volume. Horizontal bars above thick bars are one-half standard deviations. The changes between bars are significant at the 2% level where no stars are present.

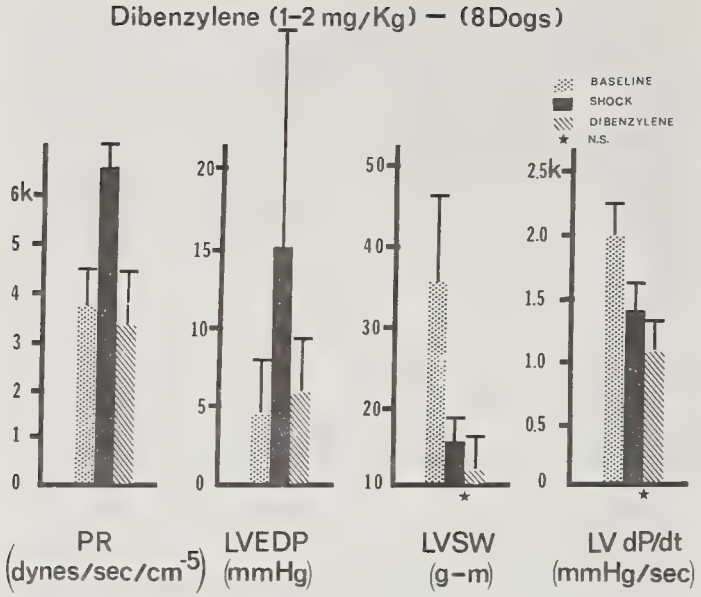


FIGURE 9.—Same as in Figure 8. PR = peripheral resistence; LVEDP = left ventricular end-diastolic pressure; LVSW = left ventricular stroke work; LV dP/dt = left ventricular dP/dt.

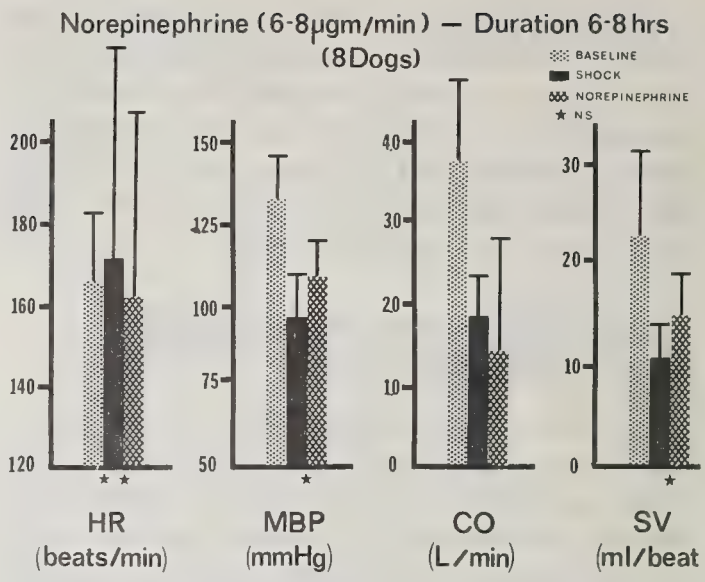


FIGURE 10.—Same as in Figure 8 for norepinephrine.

age cardiac output increased, it did not change in two animals and decreased in two. Heart rate also increased and the calculated stroke work decreased as did left ventricle dP/dt. Left ventricular end-diastolic pressure also decreased. Three of the dogs developed frank pulmonary edema within one-half hour after therapy and died. Four of the dogs died within the next 12 hours, only one dog lived beyond 48 hours, giving a 24-hour mortality of 88%.

In Group B, three subgroups were studied to test the effect of different dose levels of norepinephrine. Figures 10 and 11 show the response in eight dogs with the largest dose used, namely 6–8 μgms/min norepinephrine administration. This amount of norepinephrine slightly increased mean blood pressure and stroke volume but decreased cardiac output and peripheral resistence. Left ventricular stroke work, left ventricular end-diastolic pressure and left ventricular dP/dt max slightly increased. The same changes were present in different degrees when the animals were treated with smaller doses of norepinephrine. In the first group with 2–4 μg/min of norepinephrine, peripheral resistence actually decreased and cardiac output slightly increased. The 24-hour mortality in these animals was 50% with five out of eight surviving more than 24 hours in the group of the highest dose of norepinephrine.

In Group C, phenoxybenzamine and volume expansion was combined with norepinephrine. Again, a significant decrease in mean blood pressure and peripheral resistence occurred despite of the norepinephrine addition. The significant fall in left ventricular end-diastolic pressure was also present. Despite this, three dogs developed pulmonary edema. The 24-hour mortality was 75% in this group.

In Group D, isoproterenol alone and in combination with norepinephrine was tested follow-

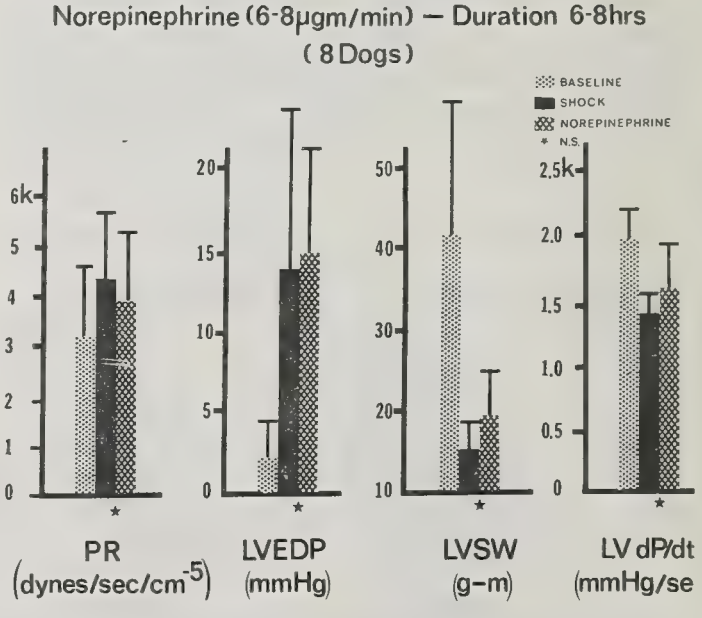


FIGURE 11.—Same as in Figure 9 for norepinephrine.

ing the development of cardiogenic shock. First, isoproterenol was given for about two hours following which no medication was given for one-half to one hour and then isoproterenol and norepinephrine were combined. Figures 12 and 13 show the results of these studies. Following administration of 2–4 μg/min of isoproterenol, there was a significant increase in heart rate and slight further decrease of mean blood pressure. Peripheral resistence also decreased while cardiac output slightly increased. Left ventricular dP/dt max significantly increased. Tachyarrhythmias and periods of ventricular tachycardia occurred during isoproterenol administration.

The combination of isoproterenol at 1 μg/min with 2–4 μg norepinephrine resulted in significant hemodynamic improvement. There was a significant increase in cardiac output and stroke volume. Left ventricular dP/dt increased more than with isoproterenol alone. Left ventricular stroke work increased while left ventricular end-diastolic pressure slightly decreased. Peripheral resistence showed a decrease with small decrease of the mean systemic pressure.

When myocardial metabolism and left ventricular end-diastolic and end-systolic volume were studied, it was found that isoproterenol alone increased excess lactate production and increased myocardial oxygen consumption (Figures 14 and 15). There was no significant decrease of the heart volumes with isoproterenol. On the other hand, a combination of isoproterenol with norepinephrine led to improvement of myocardial metabolism with decrease of myocardial excess lactate production, an increase of the ejection fraction as a result of greater decrease of end-systolic than end-diastolic volume. This reversal of myocardial anerobic metabolism occurred while left ventricular stroke work increased indicating a more efficient oxygen utilization with the combined treatment. Reversal of myocardial lactate production to lactate utilization occurred only with this combined treatment.

The drug studies indicated that the best hemodynamic results and metabolic improvement in experimental cardiogenic shock were obtained with a combination of isoproterenol and norepinephrine. This occurred when both were given at about one-half of the necessary dose to produce hemodynamic changes than when given alone. This probably was due to an additive effect requiring smaller doses of each drug in combination to exert maximum improvement.

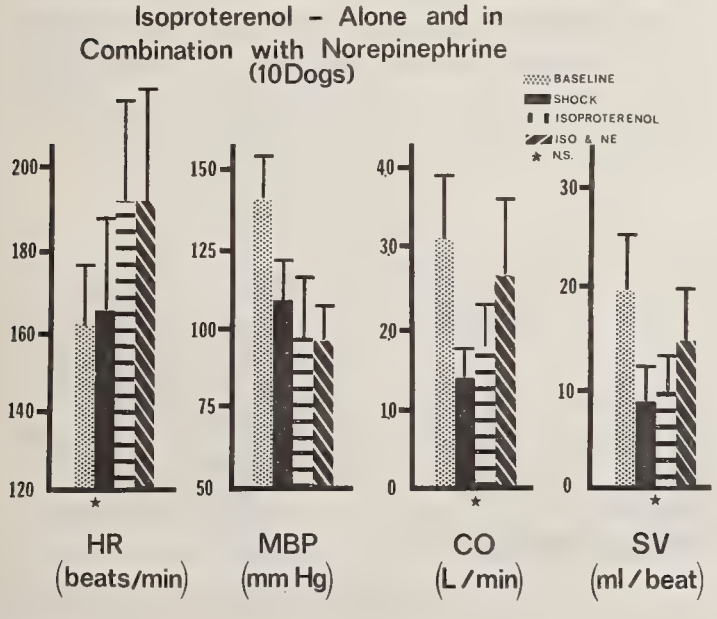


FIGURE 12.—Same as in Figure 8 for isoproterenol and for isoproterenol and norepinephrine combined. Statistical significance is calculated between shock state and treatment with isoproterenol alone and in combination with norepinephrine (not between isoproterenol and isoproterenol plus norepinephrine).

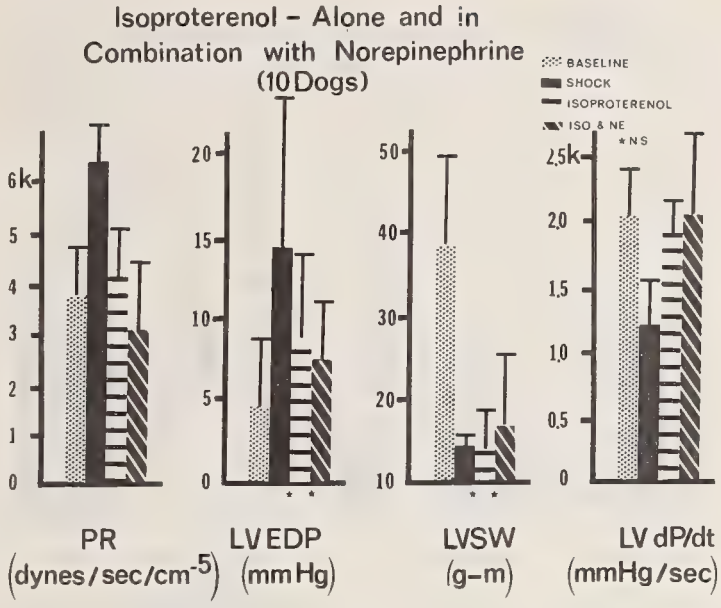


FIGURE 13.—Same as in Figures 9 and 12 for isoproterenol and in combination with norepinephrine.

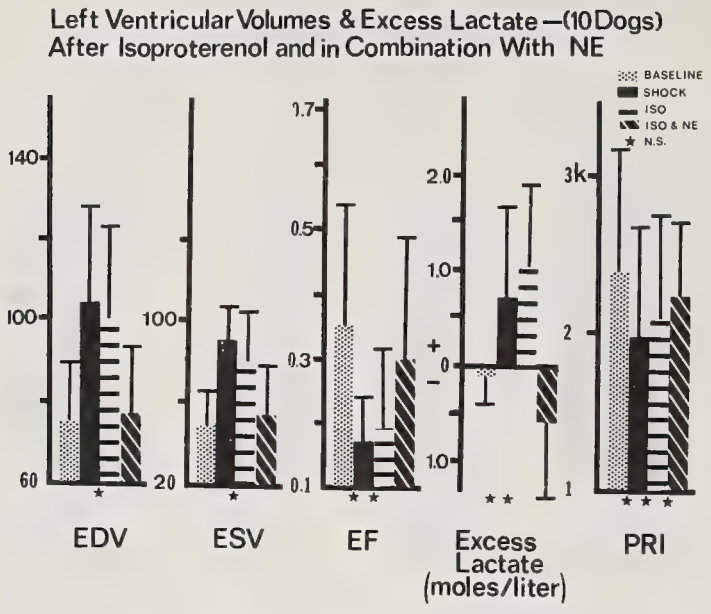


FIGURE 14.—Left ventricular end-diastolic (EDV) and end-systolic volumes (ESV); ejection fraction (EF); myocardial excess lactate and pressure rate index (PRI, modified tension time index). Note improvement of EF and excess lactate with combined treatment.

CONCLUSIONS

The total number of dogs to date in whom metallic mercury was injected into the coronary artery with the intent to produce myocardial infarction and cardiogenic shock was over 100. In 98%of the animals the circumflex or the anterior descending coronary artery could easily be catheterized with our double catheter technique. When the circumflex coronary artery was injected with mercury 18% developed ventricular fibrillation soon after injection. A small number of these could be cardioverted and the experiment could be continued. About 80% of the animals developed hypotension, decrease of cardiac output, stroke volume, and dP/dt. In 5% of these dogs the hemodynamic changes were moderate and did not fulfill the criteria of cardiogenic shock. Thus 75% of the total mercury injected animals developed cardiogenic shock with moderate hypotension, tachycardia, marked decrease of dP/dt, cardiac output, stroke volume, (40–50%) and changes in other parameters as described in the results. We can conclude that the mercury technique results in a reliable model of cardiogenic shock and is useful for various studies in the closed chest unanesthetized animal. Our results confirm the findings of Lluch et al., who also find this model very reliable.

SUMMARY

The model allowed us to study afferent vagal reflex mechanisms and drug effects in cardiogenic shock. It helped to elucidate a possible unrecognized positive feedback mechanism which may be detrimental to the already compromised circulatory system in cardiogenic shock. If vagal afferent reflexes are overly active in shock, then it is not surprising that sympathetic augmentation is effective. Since the organism itself supresses its maximum compensatory capacity by this positive feedback, a combination of alpha and beta adrenergic stimulating drugs may break the vicious cycle by restoring this maximum compensatory capacity. However, careful analysis of the different circulatory and metabolic factors is necessary to elucidate the most effective drug combination for the treatment of this delicately balanced condition. The mercury model is well suited for the analysis of these delicate balances and the study of combination drug treatment with isoproterenol and norepinephrine proved that not only the choice of medication but the careful adjustment of the dose is an important factor. Based on our animal studies we have similarly treated a small

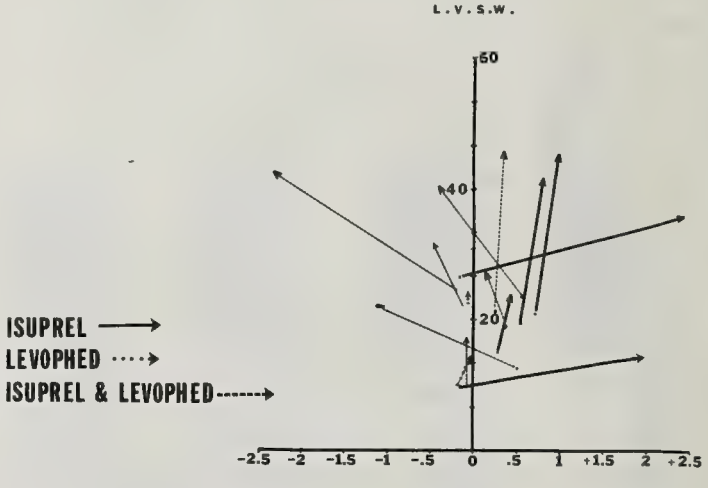


FIGURE 15.—Relationship of myocardial excess lactate to left ventricular stroke work before and during isoproterenol, norepinephrine and combined treatment in shock. Note that best effect on these parameters occurs with combined treatment.

number (5) of patients in cardiogenic shock with good results.

REFERENCES

1. AGRESS, C. M. ROSENBERG, M. J., JACOBS, H. I., BINDER, M. J., SCHNEIDERMAN, A., and CLARK, W. G. Protracted shock in the closed-chest dog following coronary embolization with graded microspheres. Am. J. Physiol. 170:536, 1952.
2. PISA, Z., and HAMMER, J. Hemodynamicke zmeny po embolizaci koronarniho reciste u psa. Cas Lek Ces 98:1344, 1959.
3. JACOBEY, J. A., TAYLOR, W. J., SMITH, G. T., GORLIN, R., and HARKEN, D. E. New therapeutic approach to acute coronary occlusion: I. Production of standardized coronary occlusion with microspheres. Am. J. Cardiol. 9:60, 1962.
4. LLUCH, S., MOGUILEVSKY, H. C., PIETRA, G., SHAFFER, A. B., HIRSCH, L. J., and FISHMAN, A. P. A reproducible model of cardiogenic shock in the dog. Circulation 39:205, 1969.
5. KORDENA, R. K., KEZDI, P., and STANLEY, E. L. A new catheter technique for producing experimental coronary thrombosis and selective visualization. Am. Heart J. (in press).
6. SHAFFER, A. B. Estimation of ventricular volumes by a constant infusion indicator dilution technique. Circulation Res. 15:168, 1964.
7. MISRA, S. N., STANLEY, E. L., and KEZDI, P. Hemodynamic determinants of cardiogenic shock. J. Surg. Oncol. 2:245, 1970.
8. KEZDI, P. and GELLER, E. Baroceptor control of post-ganglionic sympathetic nerve discharge. Am. J. Physiol. 214:426, 1968.
9. DIETZMAN, R. H., LYONS, G. W., BLOCK, J. H., and LILLEHEI, R. C. Relation of cardiac work to survival in cardiogenic shock in dogs. JAMA 199:161, 1967.

DISCUSSION

CHAIRMAN LA FARGE: Thank you, Dr. Kezdi. We have time for a brief discussion of this very provocative paper.

P. SOMANI, Abbott Laboratories, North Chicago, Ill.: You had a very good model and I'd like to see some of the data that you might have acquired but which you do not have at the moment. For a good model, I think you have to show also that some of the drugs which are clinically used are effective in your models by improving the mortality rate. I have not seen this in this morning's presentation. I'd appreciate your comment on that issue.

DR. KEZDI: Very good. We had the mortality figures; I even have a slide on the subject, but time is short. I indicated mortality with dibenzyline. In the combined norepinephrine and isoproterenol-treated animals, nine out of ten survived more than two days: obviously, the mercury erodes through the vessel wall after few days and you have to consider this when evaluating mortality. But taking 48 hours as a critical point, nine out of ten of these animals survived, which is 90% ; all other drugs resulted in a much lower survival rate.

LLOYD G. PHILLIPS, University of Minnesota, Minneapolis, Minnesota: Dr. Kezdi, did you obtain any significant immediate mortality from the primary injection of mercury?

DR. KEZDI: Yes. 18% of the injected animals developed ventricular fibrillation. A very small number (2%) could be converted and we could continue the experiment. The overall total is about 75% of the injected animals which will live and develop a good standard model of cardiogenic shock.

DR. SOMANI: Dr. Kezdi, I was quite intrigued by your results that the sympathetic amount of activity was not increased. Now the increase in sympathetic activity in any type of shock model is related to the time factor after your correction of the shock. For example, in hemorrhagic shock the sympathetic activity is initially very high but as the time goes by, the activity becomes very low. Did you find something similar in your cardiac shock model?

DR. KEZDI: We have not followed the activity longer than five hours. But there is a difference between the hemorrhagic shock and the cardiogenic shock model. In hemorrhagic shock you don't damage the heart initially at all and you have immediate baroceptor reflex compensation. Therefore you have a marked increase of sympathy activity. I assume that in the cardiogenic shock model you have immediate stimulation of the Bezold-Jarish reflex. Later on they probably adapt but after a while the decreased sympathetic activity is probably due to other factors. One of these could be the stimulation of ventricular stretch receptors. Thus, sympathetic activity is down from the onset of myocardial infarction.

CHAIRMAN: Dr. Kezdi, do you have measurements of coronary flow in response to this kind

of embolization or lactate or myocardial oxygen consumption?

DR. KEZDI: We have not done coronary flow measurements in these animals. Myocardial oxygen consumption decreased and lactate production (excess lactate) markedly increased. It was of interest to us that reversal of this did occur only with combined isoproternol and norepinephrine treatment. Also, as you have seen, the dose of both drugs was about half of that which was needed to produce changes when used alone.

BIVENTRICULAR MECHANICAL BYPASS PHYSIOLOGICAL STUDIES DURING INDUCED VENTRICULAR FAILURE

C. Grant LaFarge and W. F. Bernhard*

Effective reproducible ventricular failure models, with a low morbidity and mortality, have only recently been available for physiological evaluation of mechanical cardiac support systems. Initially, a number of methods were evaluated in this laboratory, and all rejected because they failed to meet the criteria above: serial coronary artery ligation or carbon dioxide infusion (for acute left ventricular failure); local myocardial irradiation with Strontium-90 or intoxication with cobaltous chloride(for chronic failure).

Later in our experience, two methods were developed and used successfully for evaluation of partial (left) and total (left and right) ventricular bypass systems. These studies employed pneumatically actuated, bivalved blood pumps (100 ml stroke volume) implanted and operating continuously in calves (65X90 Kg) for period up to 203 days. To produce acute left ventricular failure, a balloon cuff catheter was implanted around the main left coronary artery. At the time of physiological evaluation (12 to 28 days postoperatively) inflation of the balloon catheter produced 5–20 minutes of stable, ventricular failure. Left ventricular end-diastolic pressure returned to control values following deflation of the balloon.

For chronic irreversible ventricular failure, diffuse myocardial ischemia, and eventual fibrosis, was produced by graded infusion of latex microspheres (9 microns average diameter) into the main left coronary artery. Additionally, total cardiac failure was produced by electrically-induced ventricular fibrillation. The acute and chronic failure models proved to be effective and reproducible with low morbidity (balloon cuff) and mortality (both). In both ventricular failure and control conditions, the ventricular blood pumps proved capable of maintaining cardiac output, while reducing atrial and ventricular systolic and diastolic pressures to low normal levels (or to zero mmHg). In the balloon cuff and microsphere-induced failure states, ventricular ejection fraction improved towards normal, and abnormally elevated ventricular volumes were reduced to the normal range. No significant hematological abnormalities were produced by pumping in any of the three states.

INTRODUCTION

Effective, reproducible ventricular failure models, with a low morbidity and mortality, have long been the goal of investigators interested in the study of abnormal ventricular dynamics. The search for ideal models has recently been spurred by the development of a polyhedral family of cardiac assist and replacement devices. All of these must be evaluated in normal animals, and in animals with deranged ventricular function, prior to their human clinical application.

Heretofore, there have been few reliable ventricular failure models which could be applied to artificial heart devices. For acute left ventricular failure (1 to 24 hours), variations on a coronary constriction theme have been effective, but attended by greater than desirable mortality.[1–3]

Chronic ventricular failure models, in reliable, reproducible form, have been a real challenge, rarely met in large animals, and based on a wide variety of techniques. Examples include the Barger dog,[4] the Spann guinea pig,[5] and the Munro and Marcus dogs.[6,7] Unfortunately, small animals, including the dog, are not optimal for study of artificial heart devices: chest and heart size are much smaller, and clotting problems are monumentally greater than those of the human.

Two problems required solution: animal size, and reliable, reproducible failure states suitable for evaluation of artificial heart devices. This report is concerned with some solutions to both problems. Between the large non-human primates, the horse, the calf, the goat, and the sheep, it was quickly determined that the calf had the most numerous advantages: size, numbers available, low cost, docility, relative freedom from disease (individual and colony), and relative physiological similarity with human organ systems, including blood.

Several methods of inducing myocardial failure were tried and rejected: multiple coronary

* Children's Hospital Medical Center, Boston, Massachusetts.

ligations and carbon dioxide infusion for acute failure; Strontium-90 radiation, and cobaltous chloride intoxication, for chronic failure. The two methods which satisfied the criteria of reliability, reproducibility, and low morbidity and mortality are the central themes of this report: coronary balloon-cuff occlusion for reversible, acute myocardial failure; and coronary microsphere infusion for irreversible, chronic failure.

METHODS

Animals, Pumps, and Surgical Implantation

Calves (65–90 Kg) were selected for all studies except the Strontium-90 and cobaltous chloride experiments, in which dogs (both) and rats (cobaltous chloride) were used.

The left,[7,8] right,[9] and biventricular[10–12] pumps have been described previously in detail, as have their individual and collective implantation techniques:[7–9,11] both pumps are implanted in the left chest, as previously illustrated[10,11] (see Figure 12).



FIGURE 12.—Lateral chest X-ray of calf standing, awake, prior to onset of electrically-induced ventricular fibrillation. Head is to the right. Inflow tubes of LV (left and RV (right) pumps are shown below. Outflow pump grafts are attached above (on left) and right, to the descending aorta and pulmonary artery, respectively.

Briefly, both pumps have a stroke volume of 100 ml, with a maximum output of 11 L/min. The inflow tube is implanted in the left ventricular (LV) apex or in the right ventricular (RV) infundibulum. The outflow grafts of each pump are sutured, end-to-side, to the descending thoracic aorta, or to the main pulmonary artery. The latter is banded proximal to the graft.[9] Low-profile disc valves guard the inflow and outflow ends of the pumping chamber, which is polyurethane rubber internally coated with a matrix of flocked dacron fibrils.[11,13] Pneumatic drive lines are connected from the pumps transcutaneously to an external drive and control unit. This unit may be operated at a fixed rate, or synchronized with the electrocardiogram (Ecg) to counterpulse with the ventricle. It has a fail-safe sensor which will switch from a primary to a secondary fixed-rate unit in the event of primary failure.

Hemodynamic Studies: Cardiac Catherization and Angiography

Cardiac catherization studies were performed under a light halothane anesthesia (0.5–1.0% 2-bromo-2-chloro-1:1:1-trifluoroethane) administered through an endotracheal tube. Respirations were supported by a Bennett positive pressure ventilator, and a nasogastric decompression tube inserted. The right jugular vein and carotid artery were exposed for all studies, and cardiac catheters (United States Catheter and Instrument Corporation) positioned in RV and pulmonary artery (PA), and in LV and Aorta (Ao). The latter catheter was positioned in the main left coronary artery for induction of microsphere or carbon dioxide (CO_2) failure. An additional Statham P–866 or SF–1 catheter was positioned in the LV body for high fidelity pressure recordings. The standard protocol included baseline measurements of pressures, flows, and heart rates with pump(s) on and off, synchronous and asynchronous with the Ecg, and recordings of LV (or RV) biplane angiograms. The latter were recorded at 6 frames per second during injection of Renovist (sodium and meglumine diatrazoates), using an Elema-Schonander roll-film changer. Following this, myocardial failure would be induced by the

technique of choice, and then repeat pressures, flows, and angiograms were obtained.

High-fidelity pressures were recorded in all states on magnetic tape (Sanborn 7-channel tape recorder) at 30 i.p.s., and played back to an 8-channel Sanborn photographic recorder at 3¾ i.p.s. tape and 200 mm/sec paper speeds. The recordings were subsequently analysed on a PDP–9 computer, through an Edwin Industries X–Y digitizing table for dP/dt and dP/dt/KP (V_{ce} = velocity of contractile element shortening).[8] The simultaneously recorded angiograms were analysed on the same computer for left ventricular volumes, mass, and ejection fraction. All data were normalized (indexed) by dividing raw values by one tenth the square root of body weight in kilograms.[8,14]

Hemodynamic Studies: Awake, Rest, and Exercise

In several calves, electromagnetic flow probes (Carolina Medical Instruments) have been used to study normal resting (6) and exercising (1), and failure (1) states, including ventricular fibrillation (1). In 3 calves, indwelling PA, Ao, and LV catheters were used to monitor, continuously, the normal (3), ventricular failure (2), and ventricular fibrillation (1) states. In addition to pressure, flows were measured at rest (3), during fibrillation (1), and during graded treadmill exercise (2) by indocyanine green dye dilution cardiac output curves. For these studies, a 4-channel Sanborn stylus-recorder was used.

Other Studies: Hematology, Biochemistry, and Pathology

All animals in each category (including dogs, but excluding rats) were followed daily or biweekly with serial blood tests to determine the long term effects of pumping, as previously recorded,[10,12,13] or, in the case of the Strontium-90 dog, the effects of intracavitary, LV myocardial irradiation. In the case of ventricular failure and fibrillation, additional study was made of enzyme levels and blood gases; these entities were always normal in other experiments.

Post-mortem examinations were conducted on all animals, and on all organ systems. In the cobaltous chloride experiments, heart and organ weights were recorded. In all experiments, the heart was dissected in a standard fashion, and weighed. Samples of myocardium, and other organs, were sent for microscopic examination.

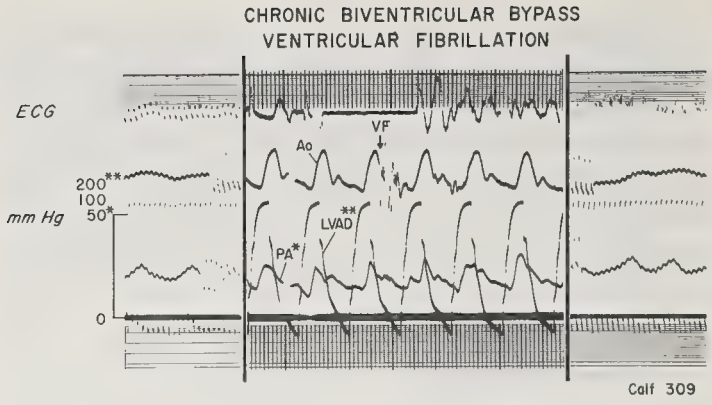
LV Failure—Acute: Coronary Ligation and Carbon Dioxide (CO_2)

Acute failure is here defined as a reversible state of short duration (less than 3 hours). Clearly, the technique of coronary ligation has been used frequently and will be reviewed by other participants in the conference.[3,15,16] Even in the best hands, the technique suffers from two drawbacks: unpredictability, and high morbidity and mortality. Our own attempts to produce reliable, acute failure with a clearly defined end-point (predetermined LV end-diastolic pressure) were, predictably, as fraught with arrhythmias and led as often to death as expected. This procedure was abandoned.

The use of CO_2 as an indicator (right atrial gas bubble to reveal the presence, radiographically, of a thickened pericardium or pericardial effusion) and its solubility in plasma suggested the possibility of acute, reversible coronary occlusion by a fine stream of bubbles directed by catheter into the left coronary distribution. Three calves were used in this evaluation: 2 controls, and 1 calf with an implanted LV bypass pump. The control calves and the pump calf were brought to the cardiac catheterization laboratory for study. Following the usual protocol, control state data were taken, including a left ventricular biplane angiogram (Figure 13). Left ventricular myocardial failure was then induced by coronary infusion of CO_2 and pressure recordings and biplane angiograms repeated. Two calves recovered, one (control) progressed after the second biplane to ventricular fibrillation. The other two returned to the animal farm for further hematologic studies.

LV Failure—Acute: Coronary Cuff

Aware of the experience of Joison in 1968, reported in 1969,[17] we adapted the balloon-cuff,



	NSR	VF
Aorta	156/120 (132)	150/120 (130)
Pulmonary Artery	26/12 (18)	30/12 (21)

FIGURE 13.—Pressure recordings obtained during a control state (left) and during induction of ventricular fibrillation (center, right). Ecg = external electrocardiogram. VF = point of electrical induction of fibrillation. Ao, PA, LVAD = aorta, pulmonary artery and left ventricular pump air drive pressures, calibrated by matching asterisks. Right and left ventricular, and right ventricular pump air drive pressures not shown. NSR = normal sinus rhythm. Mean arterial pressures appear in parentheses.

coronary occluder technique for use in inducing acute, reversible ventricular failure, rather than the chronic state which he described. The Occluder consists of a silastic balloon attached to a dacron-reinforced belt, and bonded to a long, thin silastic tube.

At the time of blood-pump implantation, the occluder was fixed around the main left coronary artery, uninflated, and the silastic tube brought out through the chest wall. Five calves were studied on 8 occasions: 7 times by cardiac catheterization and once awake, in a stall. In the latter instance, only the Ecg was recorded, prior to cardiac catheterization. In all other instances, complete cardiac catheterizations were performed: control state recordings were followed by manual inflation of the coronary occluder with CO_2 (or saline-Renovist mixture) in a syringe. Repeat pressures and angiograms were recorded after the LV end-diastolic pressure (LVedp) stabilized at the desired level. Following such hemodynamic studies (15 to 30 minutes continuous failure), the failure was reversed by deflation of the balloon, and the Ecg and pressures returned to normal (Figure 4). In all but one instance, the calves were returned, awake, to the animal farm. In the one instance, it proved impossible to deflate the balloon in any manner except by further inflation and rupture. Ventricular fibrillation followed shortly, and the animal, inadequately supported by the LV blood pump alone, died after 45 minutes.

LV Failure—Chronic: Strontium-90

Irreversible damage is done to myocardium by high-dose radiation therapy: a myocardopathy with diffuse, severe fibrosis and chronic failure results.[18] Theoretically, this effect could be reproduced experimentally by direct, specific radiation of myocardium without involvement of surrounding structures.

For this purpose, a 300-millicurie source of Strontium-90 was encapsulated and mounted at the tip of a #8 French catheter. The beta-radiation dose rate at the active surface was 10,000 rad/hr.

A 36 kilogram dog was anesthetized with sodium pentobarbital and placed on a Harvard respirator with an endotracheal tube. The special catheter was passed from the right femoral artery, retrograde, to the LV where multiple sites were selected fluoroscopically for 20 minute periods of direct beta radiation. At the conclusion of the study, the dog returned to the animal farm with no immediate change in LV pressures (110/4–8 mmHg) or Ecg. No change occurred during the subsequent 3 months. The animal was sacrificed for pathological examination at the end of that time.

LV Failure—Chronic: Cobaltous Chloride ($CoCl \cdot 6H_2O$)

An entire issue of the Canadian Medical Association Journal in 1967 was devoted to an examination of the syndrome called Quebec Beer-Drinker's Cardiomyopathy, including a review and discussion of etiology.[19] Experimental cardiomyopathy was produced by Morin in the rabbit in 1968.[20] Since this latter purpose was accomplished without additional alcohol, an experiment was designed to create cobalt cardiomyopathy in rats and dogs, prior to its use in calves.

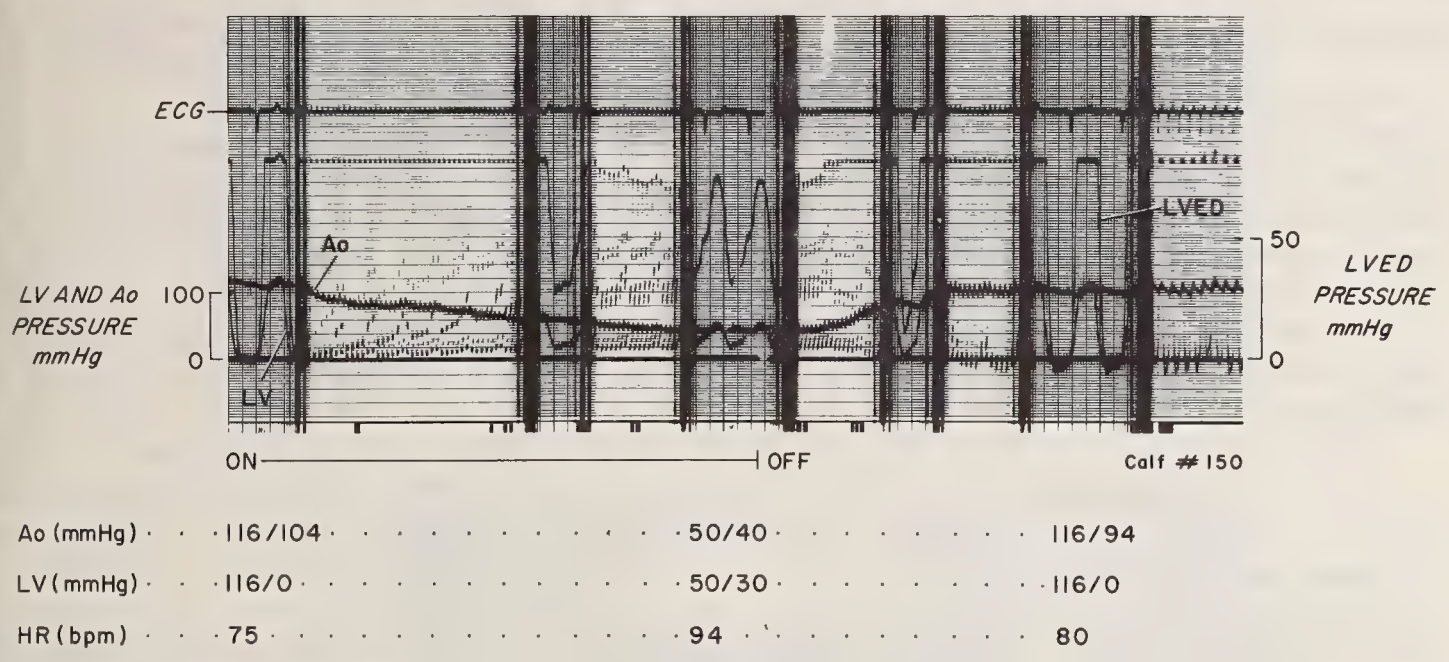


FIGURE 4.—Recordings of electrocardiogram (Ecg), aortic (Ao), and left ventricular (LV) pressures during (left) and following (right) induction of acute, reversible LV failure by inflation of a balloon cuff occluder around the left main coronary artery. HR = heart rate.

Sixteen rats were given a 2.5% solution of cobaltous chloride: 3 intra-peritoneally, 3 subcutaneously, and 6 *per os,* with 4 controls. Following this, 10 dogs were studied with the same 2.5% cobaltous chloride solution: 5 animals were injected subcutaneously with 10–20 mg/Kg daily; 2 were injected intraperitoneally with 1–2 mg/Kg daily; and 3 were given 10–20 mg/Kg *per os,* daily.

All rats, at death or sacrifice, were autopsied, and hearts dissected and weighed by standard techniques, as were the dogs'. Cardiac catheterization with angiography, under pentobarbital anesthesia, were performed in 4 dogs surviving 7 to 21 days.

LV Failure—Chronic: Microsphere Infusion

A profound state of LV myocardial failure was produced reliably and free of arrhythmias in a series of experiments in isolated, supported canine hearts reported from our laboratory.[21] The technique was adapted for calves, and has been used to induce LV failure in 2 control animals, 8 calves with LV blood pumps, and 4 calves with LV and RV blood pumps. Two additional calves were studied with RV (1) and biventricular (1) failure; the former had an LV pump alone, the latter a biventricular system.

After 8 to 44 days of continuous partial bypass, cardiac catheterization was performed. During this study, 10 to 20 ml of a 3% solution of microspheres (divinylbenzene copolymer latex, 6–14 microns in diameter) were infused into the left main coronary artery in 1 ml increments every 3 minutes. The end-point of infusion was determined by arrival of the LVedp at 30–40 mmHg (25 mmHg for controls). Subsequent hemodynamic measurements and biplane angiograms were recorded, and the animals returned to the animal farm for further study.

Subsequent studies (after 4 to 64 days of microsphere-induced failure) included repeat cardiac catheterizations (10), pressure and flow measurements in stall, awake (5), and graded treadmill exercise (3). Those animals which have been sacrificed (2 animals alive at this time, at 46 and 79 days, with LV pumps) have been autopsied. The hearts have been dissected,

weighed, and sections sent for microscopic examination.

Total Heart Failure: Ventricular Fibrillation

Ten calves have been studies during electrically induced (8) or accidental (2) ventricular fibrillation: 3 with LV, and 7 with LV and RV blood pumps. One of the latter was studied in the operating room, acutely, open-chest; the rest were studied both by cardiac catheterization (8) and awake in stall (4: 4 hours, 9 hours, 32 hours, and 17 days duration of fibrillation). Post-mortem examinations were performed.

RESULTS

LV Failure—Acute: CO_2

Carbon dioxide, while inexpensive and simple to use, proved disadvantageous for four reasons: lack of reproducibility and predictability, artifactual pressure recordings, intravascular CO_2 collection, and morbidity.

In each of the 3 calves studied, it was difficult to reach a stable end-point in LVedp. The success manifest in Figure 1, in elevation of LVedp to a frank failure level (125/0–4 mmHg to 120/26–35 mmHg) is equally not manifest in a calf given the same CO_2 dosage by a flow rate and time: Figure 2. In this latter instance, the pressure of 125/10–18 mmHg reflects minimal LV myocardial failure (left side of figure).

Artifactually influenced recordings of LV pressure were produced in 2 calves, one illustrated in Figure 1 (upper right panel, right end) by the abrupt drop in peak LV pressure from 120/26–35 to 60/35 mmHg. The reason for this is illustrated in Figure 3: the collection of CO_2 (arrows) reaching the LV cavity by way of direct LV-coronary conections and presenting a blood-CO_2 meniscus at the catheter tip. The abrupt blood-CO_2 interface in the left circumflex coronary artery (arrow, Figure 3) can only influence recovery in a negative manner. In fact, it may well have been this coronary blood-gas interface which led one of the 3 calves to ventricular fibrillation following the induction of failure, and the biplane angiogram.

LV Failure—Chronic: Strontium-90 and Cobaltous Chloride

Since neither of these two techniques proved useful, the results can be presented briefly and together.

The singular absence of response to the radiation of LV myocardium by the Strontium-90-tipped catheter, either hemodynamically or microscopically, was disappointing. Throughout the 3 month follow-up period, LV and Ao pressures remained stable, as did blood counts and enzyme levels. Microscopic slides showed no significant abnormality in any of the many areas sectioned. Minor replacement of myofibrils by fibrocytes was evident in a few areas. On the basis of this study, the technique was not pursued further, either in dogs or calves.

The outstanding deficiency of the cobaltous chloride technique was the toxicity of the drug, both in rats and in dogs. This toxicity expressed itself differently, according to the route of administration, and was unpredictable as to time of occurrence or severity, in relationship to dosage. The solution was most toxic intraperitoneally: early death (1 rat in 3; both dogs) was preceded by loss of appetite and fever (dogs). Subcutaneous administration proved less toxic, but in dogs, produced major skin sloughing in 4 of the 5. Two of the 3 rats had major skin sloughs. The solution was least toxic *per os*, but caused anorexia and profuse diarrhea at all dosage levels, in both species. In toto, 3 rats (2 intra-peritoneal, 1 subcutaneous) developed congestive heart failure, cardiomegaly and ascites, and 2 dogs (subcutaneous) showed tachypnea as well. Heart weight-to-body weight ratios were increased 15 to 25% in both species, compared to controls. The microscopic appearance of the heart muscles showed intercellular edema, hydropic degeneration, round-cell infiltrate, and hypertrophic myofibrils. Because of the unpredictable and poor results, and the toxicity, this technique, too, was abandoned.

LV Failure—Acute: Coronary Cuff

The reliability, the reversibility, and the freedom from arrhythmias which mark this technique are illustrated in Figure 4, in which, in a

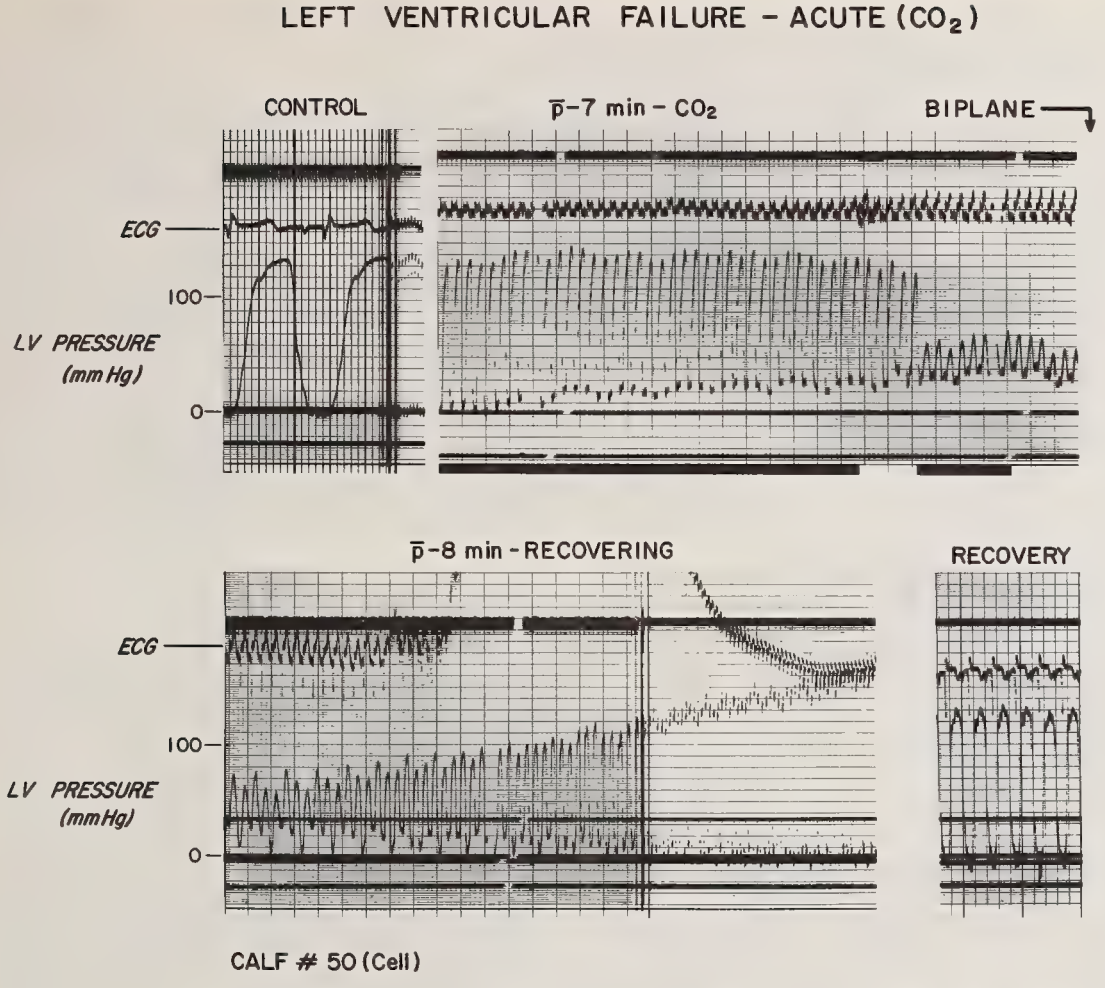


FIGURE 1.—Pressure recordings in a control calf showing: baseline left ventricular pressure (125/0–4 mmHg) and electrocardiogram (upper left); induction of failure by CO_2 infusion (120/26–35 mmHg) with marked Ecg changes (upper right); post-biplane angiogram (Figure 3) recordings of recovery phase (lower left); and, late recovery phase (130/0–5 mmHg; lower right).

control calf, the cuff was alternately inflated (on, left) and then deflated (off, center), after the steady failure state had been produced.

This technique was used in 3 calves with implanted LV blood pumps, as illustrated in Figure 5. Control LV pressure (115/8 mmHg) is altered by the coronary occlusion to 75/35 mmHg, while aortic pressure falls to 75/56 mmHg. The left ventricular quickly restores Ao pressure to 135/95 mmHg and reduces LV pressure to 30/8 mmHg.

Pressure-volume work diagrams were calculated for all 4 hemodynamic states in this calf (Figure 5): control and pump-assisted; normal and failure. Calculated from simultaneously recorded high-fidelity pressures and angiograms, the 4 work diagrams are illustrated in Figure 6. Each diagram is identified as to the state in which it was recorded, in the upper right hand corner, and the tabular data appear in Tables I and II. Similar work diagrams calculated for every calf in this group show the same effects as illustrated here, both for normal and failure states.

LV Failure—Chronic: Microspheres

The technique of producing profound myocardial failure by infusion of microspheres into the coronary artery actually provided the opportunity for the study of all physiological states desired: normal (non-failing); during the acute phase of myocardial failure; at intervals during long-term, gradually compensating failure (up

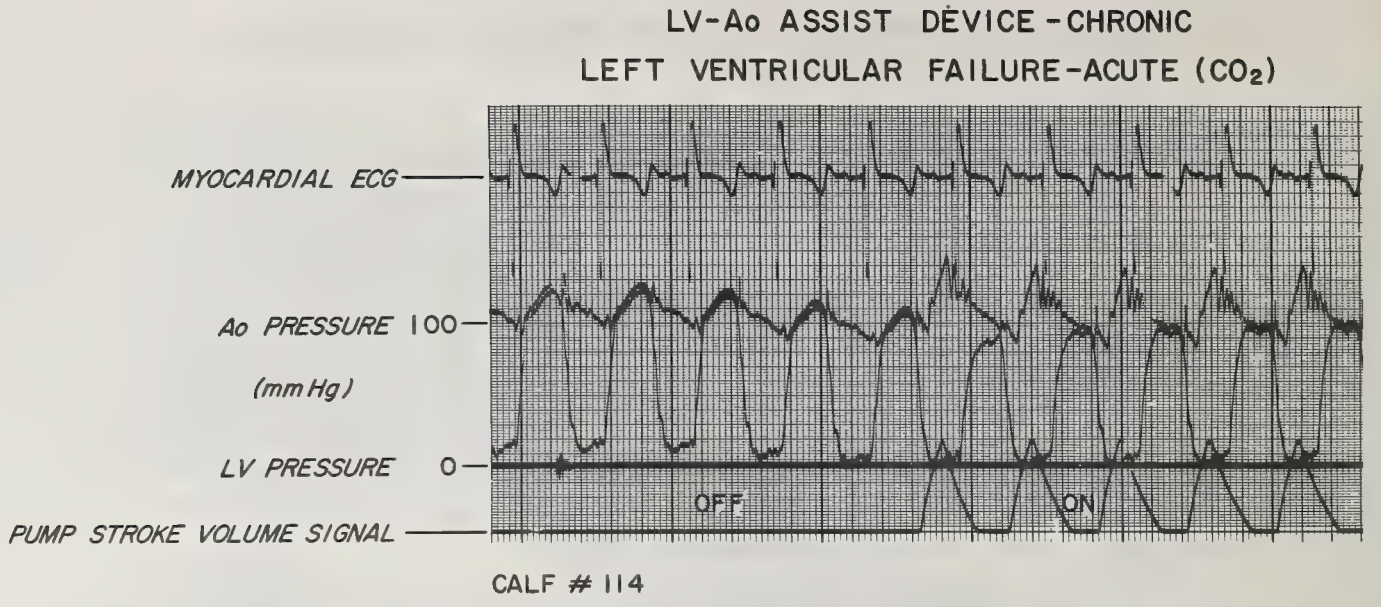


FIGURE 2.—Pressure recordings from a calf with a chronically LV blood pump, following induction of mild LV failure by coronary CO_2 infusion. Left side of figure, pump off, LV pressure 125/10–18 mmHg; right side of figure, pump on, synchronous with Ecg, LV pressure 90/2–8 mmHg. Note increase of aortic pressure from 125/100 (left) to 140/95 mmHg (right).

to 64 days); and awake, at rest in stall or during treadmill exercise.

Two controls, 8 calves with LV blood pumps and 4 animals with LV and RV pumps have been studied in this form of LV failure, with an additional 2 animals having induced RV failure. The latter 2 experiments can be dismissed summarily: right coronary microsphere infusion is lethal. The calf with the LV pump alone showed good evidence of RV failure, but developed ventricular tachycardia, episodes of bradycardia, and died on the third day after the microsphere infusion. The calf with the RV and LV pumps had microspheres infused in the left coronary artery, as usual, and one third the dose infused in the right coronary. Upon arrival at the animal farm one hour later, he was found in ventricular fibrillation. Nine hours later, his aortic pressure was steady at 125/85 mmHg. The bladder of the right pump bladder then gave evidence of rupture, and although this was not fatal, the animal was sacrificed.

The pathological results of the diffuse microsphere-induced ischemia in the distribution of the left coronary artery are illustrated in Figure 7. Small islands of normal and hypertrophied myocardium (upper left) are surrounded by widespread deterioration of myofibrillar structure, round cell infiltrate and proliferating fibroblasts and fibrocytes. Many microspheres are visible in this low-power photomicrograph of LV myocardium obtained 21 days after induction (LVedp = 30 mmHg) of failure. At sacrifice, the heart weighed 140% of comparable controls, and the LVedp was 22 mmHg.

The effect of microsphere induced failure on ventricular function is illustrated in Figure 8, where end-diastolic and end-systolic frames from biplane angiograms compare a control (above) to a failure (below) calf. The end-diastolic volumes were increased in a manner comparable to that illustrated in Figure 6, and the ejection fraction similarly reduced by microsphere infarction (normal calf 48%; failure calf 23%; Figure 8).

The hemodynamic effects of this type of failure are partially illustrated in Figures 9 and 10, two different expressions of myocardial failure: shock and pulmonary venous hypertension. Although this particular calf did not manifest clinical pulmonary edema, 4 have done so, including the calf illustrated in Figure 10. This latter recording is from the second study of a calf with an LV pump, only, obtained on the 46th day of continuous bypass. Total LV



FIGURE 3.—A lateral frame from a biplane angiogram recorded at 6 frames per second in a control calf following coronary infusion of carbon dioxide. Arrows point to areas of accumulation of CO_2, including the branches of the circumflex coronary artery.

bypass began 38 days previously with microsphere infarction and the elevation of the LVedp to 45 mmHg. By the time of this second study, the LVedp was 29 mmHg (q.v., Figure 10). At the final study, after 64 days of total bypass (72 days of pumping), the LVedp was 22 mmHg. The animal died after stepping on and kinking his air drive line, and, presumably, developing severe congestive heart failure.

Total Heart Failure: Ventricular Fibrillation

Three calves with LV blood pumps were studied during accidental (1) or electrically induced ventricular fibrillation; 7 calves with LV and RV pumps were similarly studied, 1 in accidental and the other 6 in elective fibrillation.

The calf with the LV pump fibrillated because of irreversible obstruction of the left coronary artery by the balloon cuff occluder. The calf with biventricular pumps fibrillated after left and right coronary microsphere infusion.

All animals in ventricular fibrillation demonstrated the same findings. Those with LV blood pumps alone eked out a progressively deteriorating existence from 45 minutes to 3 hours. Return of blood through the pulmonary circuit was not sufficient to supply the LV blood pump with a decent cardiac output.

Of the 6 calves who were studied with biventricular pumps, 4 had cardiac catheterization and were later studied awake in their stalls, during 4 hours, 9 hours, 32 hours, and 17 days of ventricular fibrillation. The LV and RV pump positions in the chest in these animals are il-

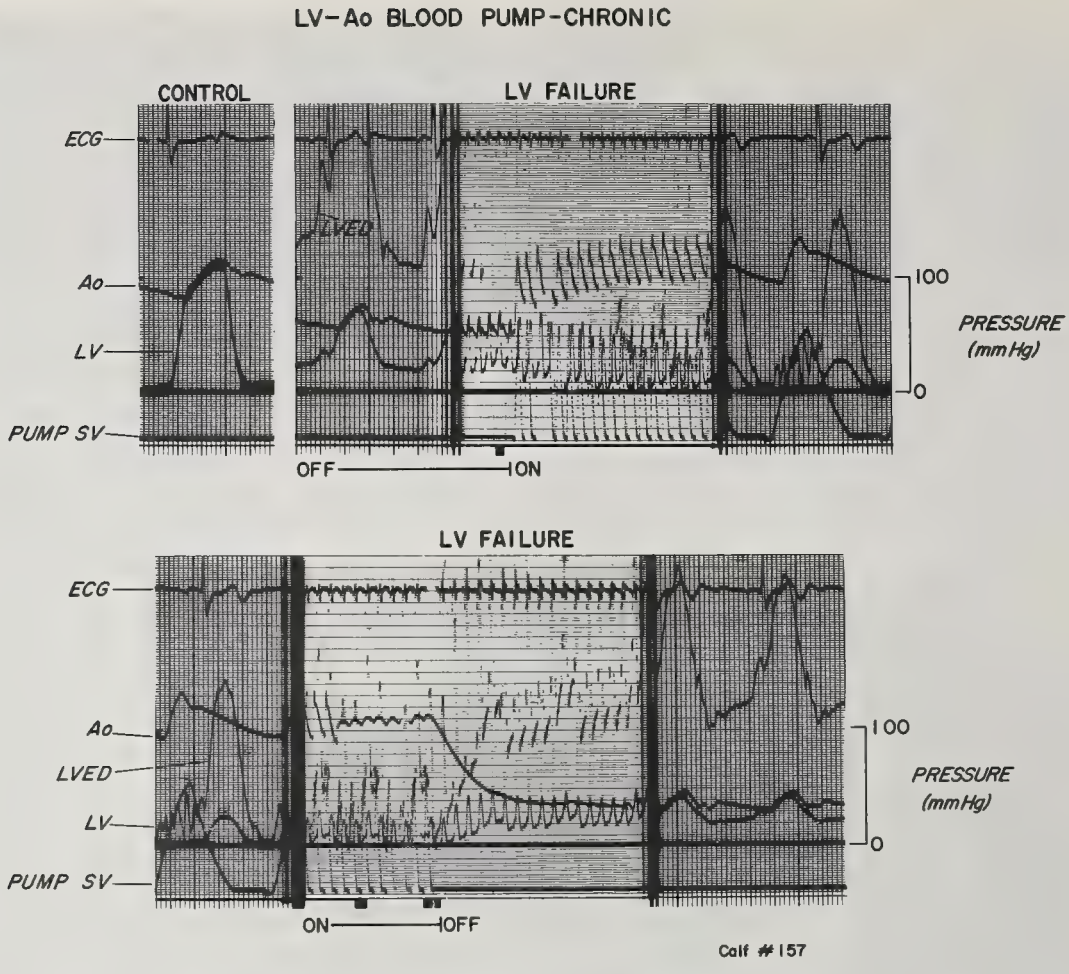


FIGURE 5.—Pressure recordings before (upper left) and during (upper right and lower panel) acute, reversible LV failure induced by inflation of a coronary balloon cuff occluder. The control state (upper left) is followed by induced failure (upper center), hemodynamically corrected by the LV blood pump (upper right, lower left). A biplane anigogram was recorded between upper and lower panels. Non-assisted pressures (lower right) following biplane confirms stability and profundity of failure state. Ao,LV = aortic and left ventricular pressures: LVed = LV end-diastolic pressure, 5× amplified; pump SV = electrical pump stroke signal (baseline = full).

lustrated in a standard chest x-ray obtained in one of these animals (32 hours), Figure 11.

Figure 12 illustrates the transition from normal sinus rhythm to ventricular fibrillation in the calf whose circulation was successfully maintained for 17 days at a cardiac output of 6.6 to 7.3 liters per minute. Pressures, likewise, remained normal, as illustrated and tabulated in Figure 12, and as measured daily throughout the 17 days. Biplane angiograms, performed after 24 hours of ventricular fibrillation confirmed the adequacy of pump maintenance of the circulation.

DISCUSSION

The principal object of this series of studies was the development of reliable techniques for the creation of stable, reproducible, acute-reversible and chronic-irreversible animal models of left ventricular myocardial failure. What concluded the search was the development of so excellent a model of acute, chronic, and compensating failure that no more useful model could be envisioned for the study of ventricular bypass pumps. All that remains is the development of a model of severe, chronic, but reversible ischemic failure: the analog of acute myocardial infarction.

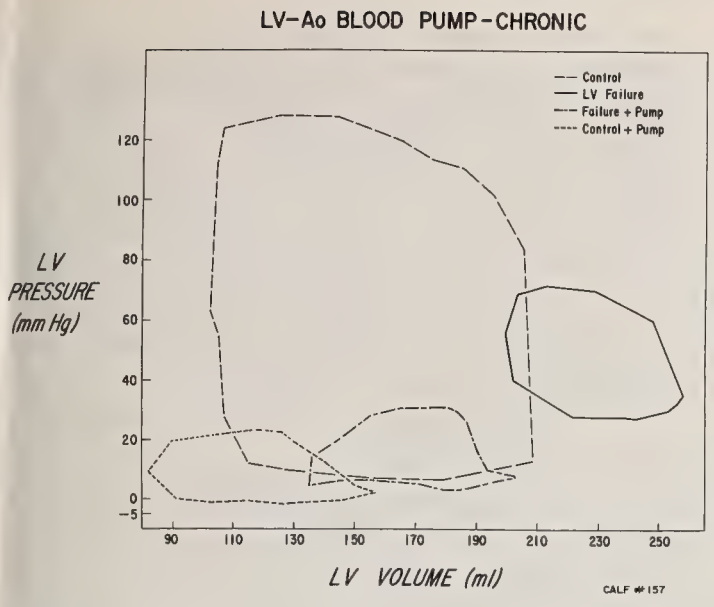


FIGURE 6.—Pressure-volume work diagrams describing the effect on LV function of blood pump assistance in control and reversible LV failure states. Corresponding numerical data are found in Tables I and II.

Initially, the search for a closed-chest, acute failure model directed us towards CO_2 infusion, since it was simple to use and reversible. Clearly, it proved hazardous generated artifacts, and proved far from reproducible. Thus, left coronary occlusion by mechanical means as the only alternative, and the balloon cuff-occluder technique proved to be very useful.

Positioned at the time of pump implantation, the balloon could later be inflated manually to produce a steady-state, arrhythmia-free level of failure at a predetermined LVedp. It proved eminently reproducible (Viz. Figure 6) in one study where, at a 1 week interval, two non-pumping failure states yielded the same data as are tabulated (singly) in Tables I and II. Similarly, from calf to calf, the same reproducibility and freedom from arrhythmias has been evident. The single disaster (balloon inflation tube kinking) produced 1½ hours of arrhyth-

TABLE I.—*Numerical data corresponding to Figure 6. The four hemodynamic states are identified in the left hand column. Left ventricular (LV) failure was induced by coronary balloon cuff occlusion. Ao, LV = aortic and LV pressures (mmHg). EDV and ESV = end-diastolic and end-systolic volumes. SV = stroke volume. E.F. = ejection fraction. Max V_{CE} = maximum velocity of contractile element shortening = dP/dt ÷ P. Max dP/dt = maximum rate of change of LV pressure during isovolumetric systole. PV work = pressure — volume work = area enclosed by the loop.*

ANIMAL & PUMP STATUS		Ao	LV	*EDV	*ESV	*SV	*E.F.	*MAX V_{CE}	*MAX dP/dT	PV WORK
		MMHG	MMHG	ML	ML	ML		SEC^{-1}	MMHG/SEC	MMHG·ML
CONTROL	OFF	136/108 (118)	136/13	209	106	103	0.49	2.2	894	10,741
	ON	150/108 (128)	23/2	157	82	75	0.48	(2.6)	800	1,251
LV FAILURE	OFF	72/57 (62)	72/37	259	200	59	0.23	0.6	342	1,940
	ON	133/101 (114)	30/6	203	135	68	0.33	(1.3)	315	1,181

* Indexed

Calf #157

TABLE II.—*Numerical data corresponding to Figure 6 and amplifying Table I. HR = heart (and pump) rates. SV = LV stroke volume. CO_{LV} = minute output of LV. PSV = pump stroke volume. CO_P = minute output of pump. CO_{ATR} = minute output from atrium in addition to CO_{LV}. CO = total cardiac output per minute: CO = CO_{LV}, non-pumping; CO = CO_{LV} + CO_{ATR} pumping.*

ANIMAL & PUMP STATUS		HR	*SV	*CO_{LV}	*PSV	*CO_P	*CO_{ATR}	*C.O.
		PER MIN	ML	L/MIN	ML	L/MIN	L/MIN	L/MIN
CONTROL	OFF	76	103	7.8	--	--	--	7.8
	ON	75	75	5.6	110	8.3	2.7	8.3
LV FAILURE	OFF	79	59	4.7	--	--	--	4.7
	ON	68	68	4.6	90	6.1	1.5	6.1

*Indexed

mia-free, stable failure (LVedp = 28 mmHg)! Over inflation and rupture of the balloon, with return of normal coronary flow, provoked a fatal series of arrhythmias ending in ventricular fibrillation.

The search for a closed-chest chronic failure model is still under way. Quebec beer-drinker's, cobalt cardiomyopathy, successfully used in rabbits[13] looked promising, but the cobaltous chloride proved so generally toxic (at any dosage level in rats and dogs) that the promise for calves remained unfulfilled. Chaga's disease was considered, but found unappealing for vectorial reasons.

The profound cardiomyopathy which accompanies radiation therapy to the myocardium[18] prompted an attempt to create specific myocardial lesions from within, using Strontium-90 (beta-radiation) to the endomyocardium. No success was achieved with this catheter technique.

Of all of the substances which have been infused into the coronary arteries and myocardium to create obstruction to flow and distal ischemia, our experiences, from 1963 on, led us finally to select the microscopic latex microspheres (6–14 microns in diameter) which have proved so useful.[21] Larger diameter microspheres are worse: in fact, the larger the diameter, the greater the frequency of disturbing arrhythmias. In the dog,[21] the small microspheres are best; in the calf, they have been nearly infallible, except for the animals with right coronary infusions. This latter fallibility is due, perhaps, to the significant influence which the beads must exert over the sino-atrial pacemaker.

In the left coronary artery, the reproducibility of a desired end-point in LVedp (30–40 mmHg), the freedom from immediate or late arrhythmias, and the prolonged state of slowly self-compensating failure have characterized an extremely desirable animal failure model. Even the method of compensation is desirable: ventricular hypertrophy with gradual return of LVedp toward—but rarely to—the normal range (0 to 16 mmHg).

As the result of a personal communication with the author, the microsphere technique described was employed at the National Heart Lung Institute in two studies: baseline failure evaluation in control animals;[23] and adaptation of this technique and coronary-cuff balloon occlusion for evaluation of intra-aortic balloon pumping systems,[24] both studies in calves. There are no essential differences between the results of those studies and those reported here and previously. However, in a bypass pump-assisted animal one can begin a prolonged period of chronic failure at a higher LVedp (non-pumping) than one can afford to do in an unassisted animal. This has proved useful in the performance of late and repeated treadmill exercise studies in animals in failure, the subject of a future report from our laboratory.

In the microsphere-induced failure state, the acute phase (first 1–3 hours), hemodynamic evaluation yields data which are exactly comparable to that produced by the reversible, balloon-cuff coronary occluder. Hence, we have abandoned the latter technique for the former, since the only remaining advantage of the occluder is the reversibility to the normal state. The only condition in which this capability would be useful is the serial study of the hemodynamic effects of pump bypass, with and without LV failure, in the long-term reduction and mainte-

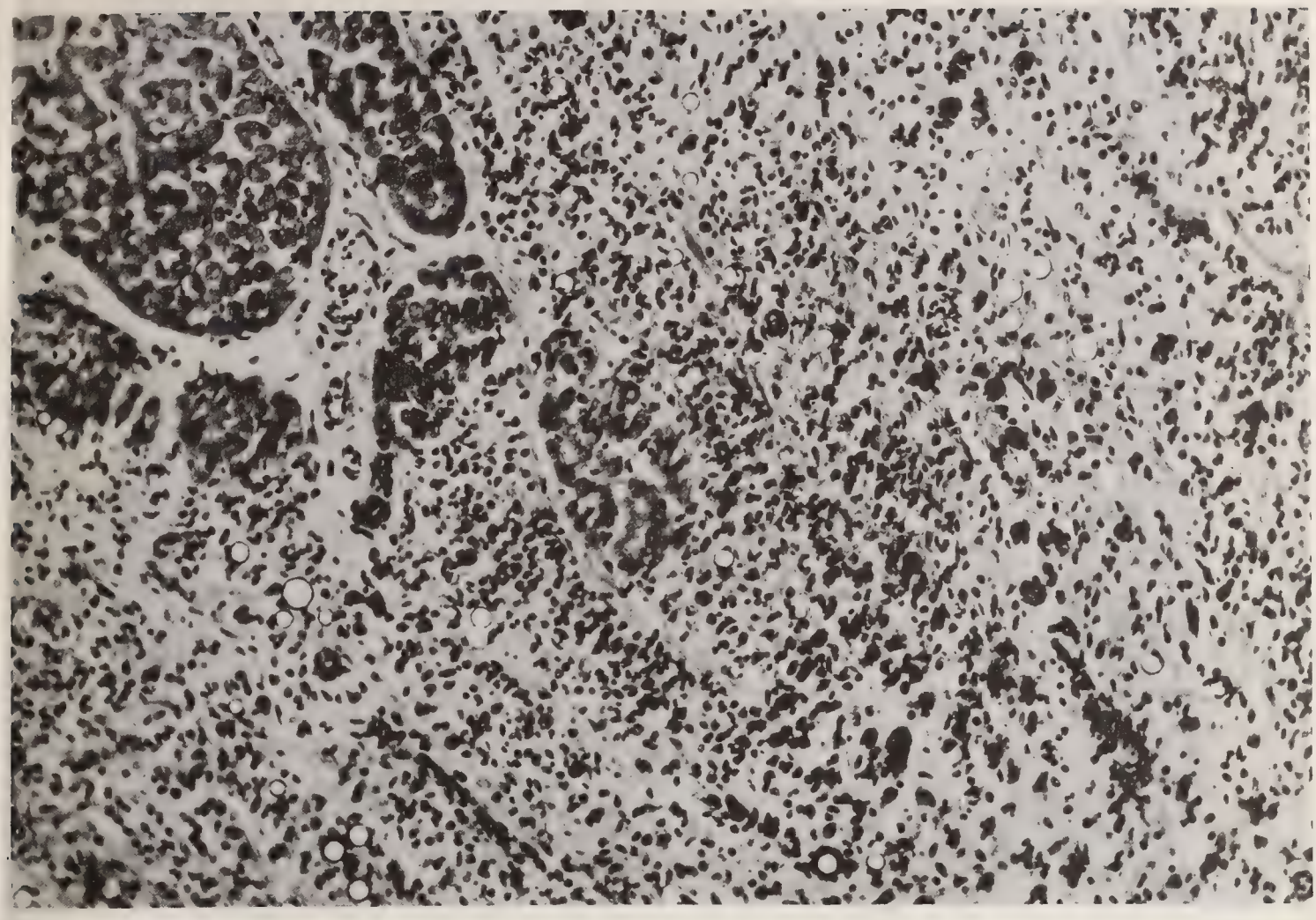


FIGURE 7.—Low power photomicrograph of myocardium from a calf 21 days after microsphere induction of diffuse, ischemic infarction. Normal and hypertrophied myocardium (upper left) is adjacent to necrosis (center) diffuse round cell infiltrate and replacement of myofibrils with fibroblasts. Latex microspheres (6–14 microns diameter) are evident.

nance of ventricular pressures at 0 mmHg.

Thus, the microsphere failure model is, so far, the one most useful for an evaluation of long-term mechanical ventricular support or bypass systems. The normal state is followed by acute failure; this, in turn, yields to a chronic failure state, gradually compensating by myocardial hypertrophy. The disadvantage of the model is that it does not resemble the principal clinical source of acute and chronic failure: major coronary artery disease. A reproducible model for that state has yet to be generated. It does, however, represent well the clinical state of diffuse, small-vessel coronary artery disease.

While the microsphere failure model has proved the most useful for hemodynamic evaluation, there is a distinct role for the total heart failure model of ventricular fibrillation. It represents the "acid test" of the combined animal-pump system: not only is the "failure" complete, but the myocardium is consuming large quantities of energy, and is in a contracted state (resembling end-systole). The latter quality provides a small end-diastolic volume for a mechanical device to draw upon, and serves as a limiting factor in total "cardiac" (pump) output. In fact, the pump must draw also upon the atrium.

One of the more fascinating observations in the studies of failure and fibrillation reported here, and previously, is the extent to which a mechanical pump, with its inflow tube in the left ventricle, draws upon the left atrium di-

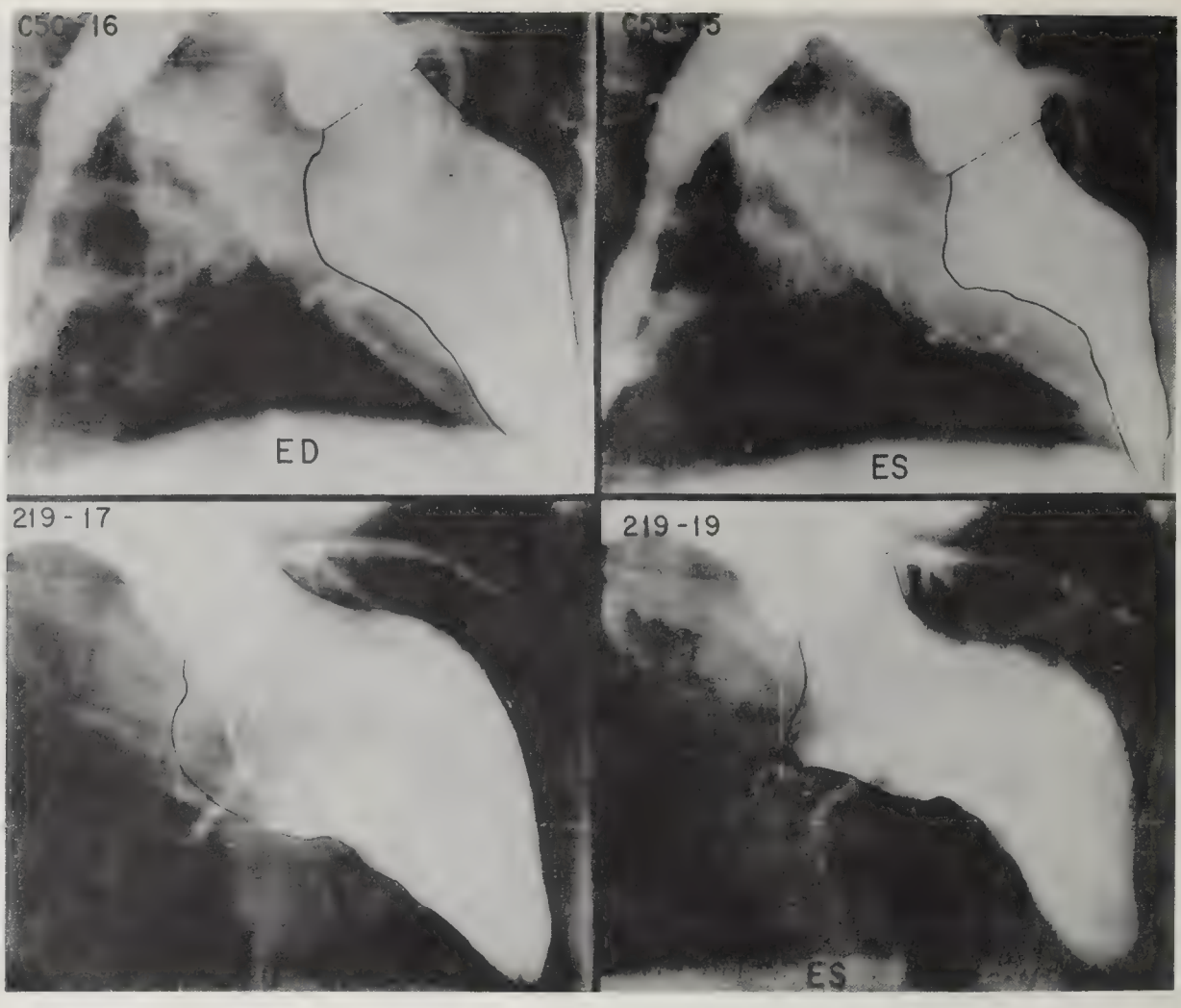


FIGURE 8.—End-diastolic (left) and end-systolic (right) frames from a biplane angiogram recorded in a normal, control calf (above; see also Figure 1), and in a calf after induction of left ventricular myocardial failure by coronary microsphere infarction. (Distortion in descending thoracic aorta, calf C–50, caused by substituted vascular prosthesis with flocked dacron fibril blood-material interface; an early study.)[21]

rectly for filling volume. The earlier inexplicability of the phenomenon of reduced ventricular stroke volume but increased "cardiac output" in the face of a closed aortic valve[22] finally yielded to the data in Table II (Figure 6).

Note the column headed COatr: it represents that fraction of total output which is not solely left ventricular (COlv), but is included in the total measured output of the ventricular bypass pump (COp). Not only in these two instances in the calf whose data are tabulated in Table II (pumping in normal and in failure states), but in almost every study of LV, RV, or biventricular pumps, the total cardiac output increased during pumping. The aortic valve being closed (note equivalent LV pressures in Table I), there are only 4 possible sources of blood for the pump: the left ventricle, the left atrium, the LV-coronary anastamoses, and the aortic end of the pump. That there is no significant retrograde flow into the pump has been proven on many occasions by electromagnetic flow probe monitoring. The volume available through the LV-coronary asastomoses is extremely small (no more than 10 ml/min). The source of the increased cardiac output must, then, be left atrial. Thus, the left ventricular blood pump tends to unload the left atrium both directly and indirectly. The same phenomenon has proven true during pure RV bypass: cardiac output in-

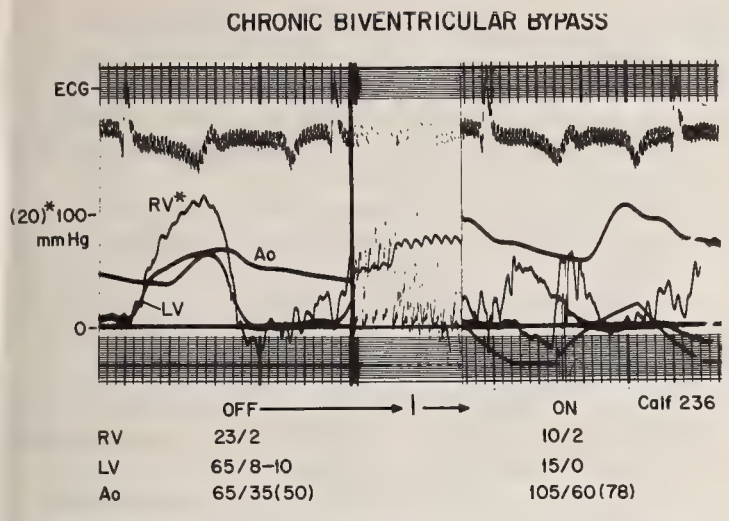


FIGURE 9.—Pressure recordings obtained after 19 days of microsphere-induced left ventricular failure. Non-pumping, control state (OFF, left) represents clinical state of cardiogenic shock. Pumping state (ON, right) demonstrates restoration of Ao pressure to normal, and reduction of RV and LV pressures to low levels, with increase in cardiac output from 4.8 to 6.1 L/min. RV,LV = right and left ventricular peak and end-diastolic pressures. Ao = aortic pressure, mean in parentheses.

creases due to an additional right atrial contribution to right ventricular pump filling.[9]

Finally, the left ventricular function data, painstakingly calculated from high-fidelity pressure recordings and biplane angiograms, are essentially the same for acute, reversible failure (Figures 1–6) as they are for the acute phase of chronic, irreversible failure: the LV end-diastolic and end-systolic volumes increase to the same extent, the ejection fraction, LV end-systolic and aortic pressures fall to the same extent, and the restoration by pumping towards normal is to the same extent (Figure 6) in both types of failure.

There are few mechanical blood pumps available today which are as capable as those reported here of reversing the failure state (to 64 days), or the total failure state (to 17 days of ventricular fibrillation), or of continuing partial bypass at 4.5 to 9 L/min for periods of time up to 203 days. The important point, however, is that this LV and RV pumping system

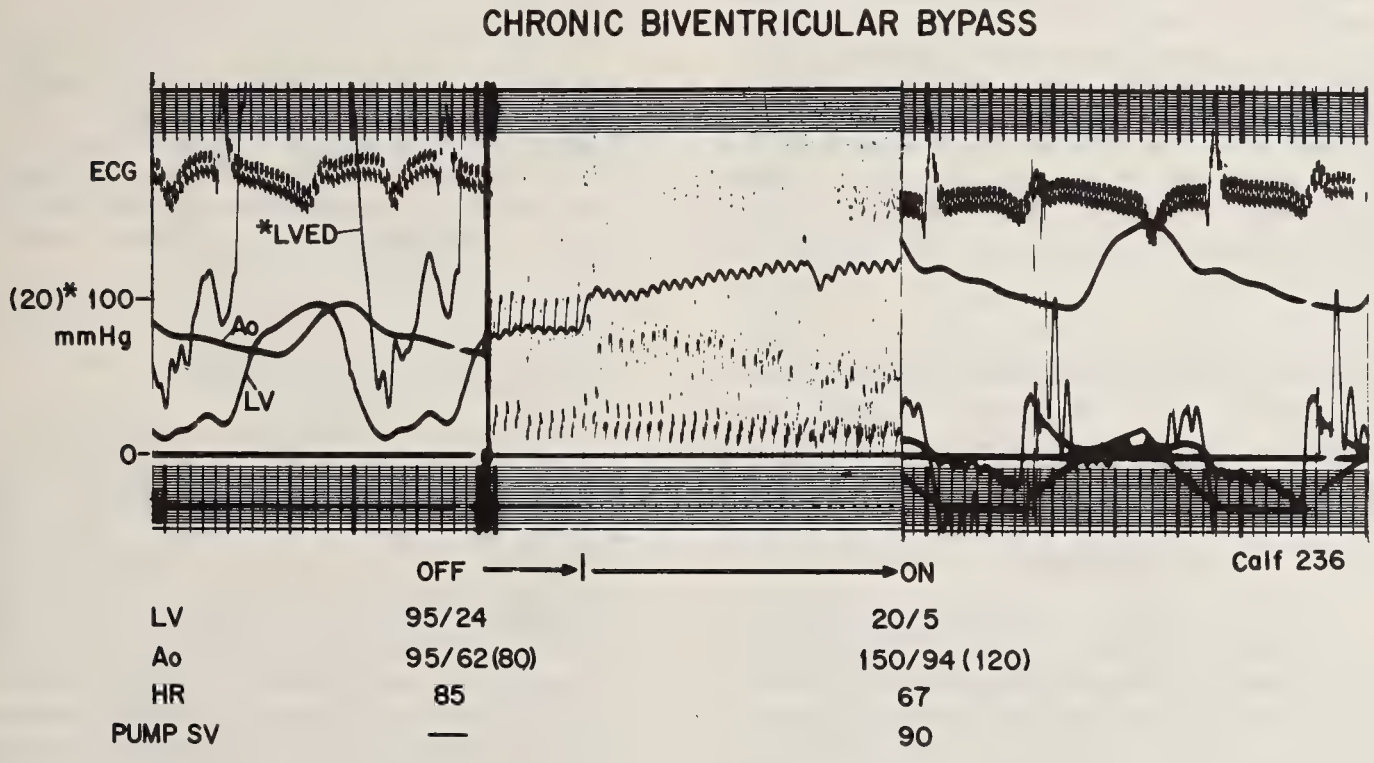
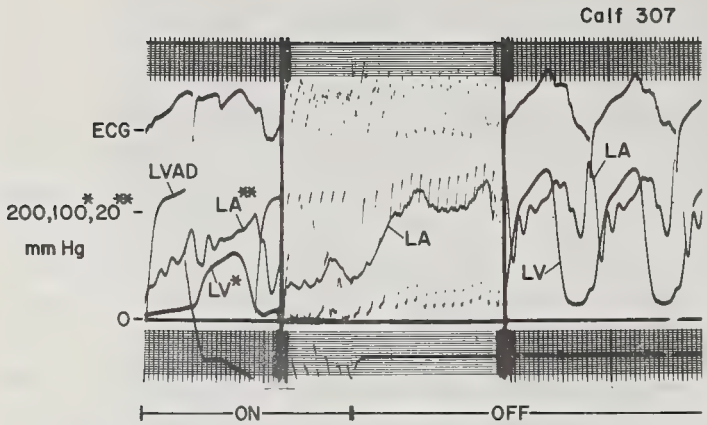


FIGURE 10.—Pressure recordings obtained after 19 days of microsphere-induced left ventricular failure. Non-pumping, control state (OFF, left) represents clinical state of pulmonary venous hypertension. Pumping state (ON, right) demonstrates restoration of aortic pressure to normal, and reduction of LV pressures to low levels, with increase in cardiac output from 5.1 to 6.0 L/min. LV = left ventricular peak and end-diastolic pressures. Ao = aortic pressure, mean in parentheses. HR = heart (and pump) rates. Pump SV = pump stroke volume.



	ON	OFF
LA	a=12	a=28
	v=16	v=26
	m=6	m=21
LV	62/11	136/29
Ao	190/136(156)	136/116(122)
RV	24/3	28/4
PA	22/14(18)	27/16(24)
HR	56	77

FIGURE 11.—Pressure recordings obtained after 64 days of partial bypass, 38 days of total LV bypass. Initial LV end-diastolic pressure 45 mmHg. Pumping (total bypass) state (left) is illustrated, with pressures from this and concomitant recordings tabulated below. Non-pumping, severe failure state (right) illustrates pressures immediately prior to the onset of pulmonary edema with rales. Restoration of pumping reversed this undesirable state. Ecg = external cardiogram (artifact from transducer-tip catheter); LA = left atrium with "a," "v," and mean pressures; LV, RV = left and right ventricular peak and end-diastolic pressures. Ao, PA = aorta, pulmonary artery pressures, means in parentheses; HR = heart (and pump) rate.

has had a challenging evaluation in the form of an animal model of ventricular failure which has no more severe or challenging equivalent in clinical human disease.

REFERENCES

1. HOOD, W. B., JOISON, J., KAMURA, R., KATAYAMA, I., NEIMAN, R. S., and NORMAN, J. C. Experimental myocardial infarction. I. Production of left ventricular failure by gradual coronary occlusion in intact conscious dogs. Cardiovasc. Res., 4:73–83, 1970.
2. STANLEY, E. L., KEZDI, P., and KORDENAT, R. K. Experimental myocardial infarction by closed chest technique. Proceedings, National Conference on Research Animals in Medicine, 1972 (in press).
3. ROSENSWEIG, J. and CHATTERJEE, S. Canine coronary insufficiency models for evaluating septems for circulatory support. Proceedings, National Conference on Research Animals in Medicine, 1972 (in press).
4. BARGER, A. C., RICHARDSON, G. S., and ROE, B. B. A method for producing chronic cardiac failure in dogs. Proc. Soc. Exper. Biol. and Med., 73:113–116, 1950.
5. SPANN, J. F., JR., CHIDSEY, C. A., POOL, P. E., and BROWNWALD, E. Mechanism of norepinephrine depletion in experimental heart failure produced by aortic constriction in the guinea pig. Circulation Res., 17:312–21, 1965.
6. MUNRO, G., BALCHUM, O. J., OWENS, J. C., and SWAN, J. Experimental chronic myocardial insufficiency produced by coronary artery embolization. Surg. Forum, 6:204–207, 1956.
7. MARCUS, E., KATZ, L. N., PICK, R., and STAMLER, J. The production of myocardial infarction, chronic coronary insufficiency and chronic coronary heart disease in the dog. Acta Cardiologica, 13:190–198, 1958.
8. BERNHARD, W. F., LAFARGE, C. G., KITRILAKIS, S. S., and ROBINSON, T. C. Development and evaluation of a left ventricular-aortic assist device. Proc. Art. Ht. Prog. Conf., 559–579, 1969.
9. BERNHARD, W. F., LAFARGE, C. G., HUSAIN, M., YAMAMURA, N., and ROBINSON, R. C. Physiologic observations during partial and total left heart bypass. J. Thorac. Cardiovasc. Surg., 60:807–817, 1970.
10. LAFARGE, C. G., and BERNHARD, W. F. Physiological evaluation of chronic right ventricular bypass. Circulation, 43–44:I–90–95, 1971.
11. BERNHARD, W. F., LAFARGE, C. G., BANKOLE, M. A., and BORNHORST, W. Chronic bi-ventricular bypass: physiological studies during induced ventricular failure and fibrillation. J. Thorac. Cardiovasc. Surg., 62:859–868, 1971.
12. BERNHARD, W. F., BANKOLE, M. A., LAFARGE, C. G., BORNHORST, W., and FRIEZE, S. Development of an experimental model for functional cardiac replacement. Surg., 70:205–214, 1971.
13. LAFARGE, C. G., CARR, F. E., COLEMAN, S., and BERNHARD, W. F. The maintenance of circulatory competence during chronic, microsphere-induced myocardial failure. Circulation, (in press), 1972.
14. BERNHARD, W. F., BUTTON, L., ROBINSON, T. C., KITRILAKIS, S., and LAFARGE, C. G. An appraisal of blood trauma and the blood material interface following prolonged assisted circulation. J. Thorac. Cardiovasc. Surg., 58:801–810, 1969.
15. LAFARGE, C. G., HUSAIN, M., ONO, K., ROBINSON, T. C., and BERNHARD, W. F. Biplane angiographic evaluation of chronically implanted left ventricular-aortic assist devices. Trans. Amer. Soc. Artif. Int. Organs, 15:454–459, 1969.
16. MALINOW, M. R., HILL, J. D., LEES, M. H., MCNULTY, W. P., and OCHSNER, A. J., III. Experimental myocardial infarction in non-human primates. Proceedings, National Conference on Research Animals in Medicine, 1972 (in press).

17. Hood, W. B. and Norman, J. C. Animal models of myocardial infarction: current limitations and future promise. Proc. Art. Ht. Prog. Conf., 559–579, 1969.
18. Joison, J., Kumar, R., Hood, W. B., Jr., and Norman, J. C. An implantable system for producing left ventricular failure for circulatory-assist device evaluation. Trans. Amer. Soc. Artif. Int. Organs, 15:417–423, 1969.
19. Stewart, J. R., Cohn, K. E., Fajardo, L. F., Hancock, E. W., and Kaplan, H. S. Radiation-induced heart disease: a study of twenty-five patients. Radiology, 89:302–310, 1967.
20. Morin, Y. and Daniel, P. Quebec beer-drinkers cardiomyopathy: etiological considerations. Canad. Med. Ass. J., 97:926–928, 1967.
21. Hall, J. L., and Smith, E. B. Cobalt heart disease: an electron microscopic and histochemical study in the rabbit. Arch. Path., 86:403–412, 1968.
22. Monroe, R. G., LaFarge, C. G., Gamble, W. J., Kumar, A. E., and Manosek, F. J. Left ventricular performance and coronary flow following coronary embolization with plastic microspheres. J. Clin. Inv., 50:1656–1665, 1971.
23. Weber, K. T., Malinin, T. I., Dennison, B. H., Fuqua, J. M., Jr., Speaker, D. M., and Hastings, F. W. Experimental myocardial ischemia and infarction: production of diffuse myocardial lesions in unanesthetized calves. Amer. J. of Card. (in press), 1972.
24. Weber, K. T., Malinin, T. I., Heck, F. J., Dennison, B. H., and Hastings, F. W. Experimental production of vascular and microvascular myocardial ischemia and infarction: the effects of different rates and degrees of coronary flow reduction in calves. Proc. Conference "Reversibility of cellular injury due to inadequate perfusion." C. C. Thomas, 1972.

DISCUSSION

Chairman P. Kezdi: Thank you Dr. La Farge. This was a beautiful presentation of the acute and chronic effect of assist devices in failure.

K. T. Weber, Jr., University of Alabama: As you know, we've utilized the same technique in conscious animals followed up to 28 days with microspheres, 6 to 14 mil in diameter. I would like to make several points. One is that even though we do get compensatory changes, i.e., changes in left ventricular wall thickness and dilatation up to 65 and 45%, respectively, one can get failure expressed under angiotensin stress or at rest, depending on the microsphere dosage, chronically elevated end diastolic pressures, reduction in cardiac index of 30%, etc. Another point is that when the same dose of microspheres is injected acutely over fifteen minutes, we can produce cardiogenic shock, as opposed to our four to six hour infusion period, which gives us the stable diffuse ventricular failure.

Dr. La Farge: I'll make two comments on that. When I first gave Frank Hastings this technique (and I might add that Dr. Weber's beautiful work is in the last two references in my paper), it was perfectly clear that the results needed to be assessed in normal animals not controlled with a pump. I've done a few normal animals, although nowhere near as many as you have. I have found that in the normal animals you can only go to an end-diastolic pressure of around 25 or 26 or you get into trouble by either the acute infusion, which I use over about a half-hour period, or the longer term infusion. I can get away with the kind of thing I talked about, like putting an end diastolic pressure of 45 mm of mercury because I have always that magic way of reversing it.

Dr. M. Klain, Cleveland Clinic, Cleveland, Ohio: I would like to comment on the coronary flow in coronary obstruction. We measured the oxygen tension in various areas of the myocardium during the obstruction. We found that immediately adjacent to the obstruction in the other areas of the myocardium there was an increase of myocardial flow. My question is: did you see any fibrillation after release of the obstruction? We have seen, especially in dogs, that after you relieve the temporary obstruction you very often obtain fibrillation in about twenty minutes. We were also able to decrease this fibrillation, as well as the fibrillation after obstruction, significantly by injecting xylocaine before the obstruction is produced.

Dr. La Farge: The answer to your question is simply no; we never had any trouble. I think the difference between my technique and Dr. Hood's and Norman's is that I never completely obstructed the left coronary artery. It is a partial, almost complete obstruction and that just may make the difference. The only fibrillation that I ever saw was in an animal whose biplane I showed with the coronary cuff. There

was a kink in the inflation tube and I couldn't deflate the cuff. From that I got a chance to study an animal over one and a half hours with a totally arrhythmia-free failure at a stable level. It was a very nice opportunity. However, in order to deflate the balloon, I had to rupture it by further inflation, which led to a wild series of arrhythmias, fibrillation and death, as you'd expect. This gave me the opportunity to study an effective unilateral pumping as well, with left ventricular bypass alone, in a state of ventricular fibrillation. I could support this animal at marginal levels for three hours and then it deteriorated.

MODEL OF ADVANCED CORONARY ATHEROSCLEROSIS, MYOCARDIAL INFARCTION AND SUDDEN DEATH WITH LETHAL ARRHYTHMIAS IN SWINE

K. T. Lee, J. Jarmolych, D. N. Kim and W. M. Lee*

The purpose of this study was to develop a model in swine for advanced coronary atherosclerosis and myocardial infarction. The approach was to combine a number of techniques that were thought to induce the development of atherosclerosis, including high-fat, high cholesterol diet plus propylthiouracil and x-irradiation to the precordial region. The dose of x-irradiation that was used produced minimal changes by itself, but seemed mainly to enhance the effect of atherogenic diets. In series I among 28 swine fed the severe atherogenic diet and x-irradiated twice, 24 developed myocardial infarcts. All had advanced coronary atherosclerosis. Twenty-two died "suddenly" in the sense that no clinical signs of illness were observed prior to death. In series II among 33 swine fed the moderate atherogenic diet and x-irradiated twice, 29 died suddenly. All had advanced coronary atherosclerosis, and 25 had myocardial infarcts. Terminal ECGs taken on 7 swine showed either ventricular fibrillation or ventricular asystole. Among 32 swine fed the severe diet and x-irradiated twice, 28 died suddenly. All had severe coronary atherosclerosis, and 26 had myocardial infarcts. Terminal ECGs taken in 9 swine showed either ventricular fibrillation or ventricular asystole.

INTRODUCTION**

A moderate degree of coronary atherosclerosis can be produced in swine by feeding a simple semi-synthetic diet containing a large amount of cholesterol.[1] More severe lesions can be produced if bile salts and propylthiouracil are added to the diet containing cholesterol. Even with these additives, a year or more is required to produce advanced atherosclerotic lesions with significant luminal narrowing.[2] In swine examined in previous experiments these slowly developing coronary arterial lesions have not been associated with myocardial infarction, sudden death or other manifestations of coronary heart disease.

The purpose of the current study was to develop a model in swine for advanced coronary atherosclerosis with its complications similar to those seen in man. Such a large animal model is presently needed for the scientific trial of therapeutic devices and surgical procedures prior to their use in man. Since the object was to develop a model for study of effects of advanced atherosclerosis, it was not considered essential that the etiology and pathogenesis of the lesion be precisely the same as in man. It was only necessary that the induced disease have clinical and anatomic features reasonably similar to those in man. For the model to be of maximum usefulness, it should be possible to produce advanced lesions in a reasonably short time. The approach chosen in the current study was to combine a number of techniques thought to enhance the development of atheroclerosis, including high-fat, high-cholesterol, bile salts, propylthiouracil and x-irradiation. It is well known that the effect of cholesterol feeding on arteries can be intensified by x-irradiation. In earlier studies with other species we have shown that intensification can be accomplished with doses of x-ray that produce little effect on arteries when given without cholesterol feeding. Using this approach, we have been able to rapidly produce occlusive coronary artery disease with myocardial infarction and sudden death in swine by the combined techniques of atherogenic diet and x-irradiation.

MATERIAL AND METHODS

The current study has been carried out in two series. Series I has been completed[3] and Series II is in progress. Male miniature swine of Pitman-Moore strain were used. The number of swine used for Series I was 100 and for Series II was 80. All were weanlings weighing 8–10 kg at the outset of the experiments. Table I shows the composition of three types of atherogenic

* Department of Pathology, Albany Medical College and VA Hospital, Albany, New York

** Supported by NIH Contract 69–2050 and HE 14177

TABLE I.—*Composition of Diets Consumed per Day in Grams*

Ingredients	"Stock" (PS)	"Mild" (PSC)	"Severe" (TSC)	"Moderate" (SC)
Casein	143	143	95	152
Sucrose	227	227	140	224
Butter	57	57	87	139
Peanut Oil	57	57	87	139
Salt Mix, Wesson	30	30	30	48
Vitamin Mix	12.5	12.5	12.5	20
Choline Chloride	2.5	2.5	2.5	4
Cellulose	121	113	24.5	41
Cholesterol	0	8	14	22
Sodium Cholate	0	0	7	11
Propylthiouracil	0	0	0.5	0
Total	650	650	500	800
Calories	2,506	2,506	2,506	4,006

diets and a stock diet used and also the amounts of each ingredient consumed per day. When the severe (TSC) diet was used, it was mixed with commercial mash for the first three weeks of a conditioning period in the following proportions: first week ¼ TSC, ¾ mash; second week ½ TSC, ½ mash; third week ¾ TSC, ¼ mash; fourth week and thereafter, full TSC diet. During the course of the experiment when any swine appeared weak and less active than usual, a portion of the TSC diet was replaced with mash for few days until the swine recovered and regained his strength.

X-irradiation of 1,500 r was given to the precordial region of pentobarbital sodium anesthetized swine twice at 4–7 week intervals (Tables II–V) by using a ^{60}Co-teletherapy unit at a distance of 60 cm with an 8.7 cm square portal. In early experiments, several months on the diet elapsed before x-irradiation was given. In later experiments (Series II), we have found 8 weeks

TABLE II.—*Data Concerning Swine Fed Severe Altherogenic Diet and X-irradiated Twice, Surviving Until Termination of Experiment*

Pig No. TSC	On diet before 1st x-ray wks.	Interval between x-rays wks.	Survived after 1st x-ray wks.	Weight, kg		Serum Cholesterol mg%		Coronary Arteries			Infarct	
				Init.	Final	Init.	Final	Avg. luminal diam. mm	Avg. wall thickness mm	Virtual or total occlusion	Rec.*	Old
1	4	4	12	15.5	—	—	247	.80	.23			
2	8	7	28	23.4	33.9	55	209	.58	.48	+		+
3	29	6	11.5	15.5	26.4	159	1176	.40	.73	+	+	+
4	39	4	9	9.1	24.5	114	532	.63	.45	+	+	+
5	40.5	6	15.5	15.9	26.7	59	1300	.73	.43			
6	40.5	6	15.5	16.5	—	120	739	.58	.53	+	+	+
7	40.5	6	15.5	—	—	150	543	.73	.30			+
8	40.5	6	15.5	21.3	38.4	89	1119	.33	.68	+		+
9	40.5	6	14	15.7	57	98	668	.70	.50	+	+	+
10	40.5	6	17	18	65	109	678	.58	.35	+		+

* Recent infarct.

TABLE III.—*Data Concerning Swine Fed Severe Atherogenic Diet and X-irradiated Twice, Dying Suddenly Prior to Termination of Experiment*

Pig No. TSC	On diet before 1st x-ray wks.	Interval between x-rays wks.	Survived after 1st x-ray wks.	Weight, kg		Serum Cholesterol, mg%		Coronary Arteries			Infarct	
				Init.	Final	Init.	Final	Avg. Luminal diam. mm	Avg. wall thickness mm	virtual or total occlusion	Rec.*	Old
11	10	6	11	16.8	15.9	98	1136	—	—	+	+	+
12	4	7	17	18.2	23.6	81	1620	.43	.55	+		
13	8	7	16	20	36.8	95	3020	.50	.75	+	+	+
14	10	6	10	23.5	30	88	1740	.63	.38	+	+	
15	10	6	12	16.1	31.4	68	1400	.40	.68	+	+	+
16	8	7	14	15.2	30	95	1840	.25	.90	+	+	+
17	29	6	8	13.2	22.5	106	1265	.23	.65	+	+	+
18	29	6	9.5	12.3	18.8	122	1382	.65	.60	+	+	+
19	30	5	10	10.2	34.1	81	1195	.53	.60	+	+	+
20	46.5	4	4.5	29.3	37	94	1380	.93	.30			
21	40.5	4	13	29.5	52.5	65	618	.65	.65	+	+	
22	40.5	6	15	24.5	40.5	95	654	.93	.28			+
23	39	6	16	8	13.6	105	951	.73	.23			+
24	40.5	6	16	16	72	133	584	.58	.65	+	+	+
25	40.5	6	18	25	63.5	90	586	.98	.35		+	
26	40.5	6	18	25	72.8	103	1149	80	.30		+	+
27	40.5	6	18.5	22	35.5	99	1597	.70	.63	+	+	+
28	40.5	6	18.5	14.5	21.6	95	977	.53	.48	+	+	

* Recent infarct.

TABLE IV.—*Data Concerning Swine Fed Mild Atherogenic Diet and X-irradiated Twice*

Pig No. PSC	On diet before 1st x-ray wks.	Interval between x-rays wks.	Survived after 1st x-ray wks.	Weight, kg		Serum Cholesterol mg%		Coronary Arteries			Infarct	
				Init.	Final	Init.	Final	Avg. luminal diam. mm.	Avg. wall thickness mm	Virtual or total occlusion	Rec.*	Old
1	7	4	4	5	10.6	85	132	—	—			
2	7	4	4	5.4	10.8	90	100	—	—			
3	7	4	4	13.2	16.6	105	165	.85	.13			
4	7	4	4	4.8	9.5	100	179	.78	.13			
5	7	4	4.5	15	20	95	181	.93	.18			
6	7	4	4.5	8.1	14.1	98	158	.80	.13			
7	7	4	4.5	10	25.5	110	153	.78	.15			
8	7.5	4	4.5	9.1	16.3	108	246	.93	.13			
9	7.5	4	4.5	13.6	—	84	149	.85	.18			
10	7.5	4	4.5	5.9	10.6	114	213	—	—			
11	7.5	4	5	11.8	14.7	80	135	.78	.15			
12	7.5	4	9	13	19.7	95	227	.50	.25			+
13	7.5	4	10	14.1	21.1	110	315	—	—		+	+
14	7.5	4	10	17.3	30	85	142	.40	.65		+	

* Recent infarct.

TABLE V.—*Data Concerning Swine Fed Stock Diet and X-irradiated Twice*

Pig No. PS	On diet before 1st x-ray wks.	Interval between x-rays wks.	Survived after 1st x-ray wks.	Weight, kg		Serum Cholesterol mg%		Coronary Arteries			Infarct	
				Init.	Final	Init.	Final	Avg. luminal diam. mm	Avg. wall thickness mm	Virtual or total occlusion	Rec.	Old
1	12	6	9	20.4	35.9	95	152	1.28	.18			
2	12	6	10	17	34.5	56	154	1.03	.23			
3	12	6	10	24.7	31.6	84	162	1.18	.23			
4	12	6	18	11.6	30	94	98	1.05	.25			

* Recent infarct.

on diet before x-irradiation to give satisfactory results.

The hearts were examined systematically, both grossly and microscopically, for infarcts or other abnormalities. After fixation in 10% formalin, the amount and severity of coronary atherosclerosis were measured by ocular micrometer.[4] In brief, the procedure for the examination of the coronary arteries consists of a series of cross-sectional cuts along the course of the three main vessels. The cuts are made at 5 mm intervals until an external diameter of less than 1 mm is reached. Alternate 5 mm segments of vessel are then removed (Figures 1 and 2), processed, and microscopic sections are prepared. Diameter of the artery, diameter of lumen, and wall thickness in its thickest and thinnest areas were measured. Average diameter of the lumen and average wall thickness were then calculated for all three main arteries.

RESULTS—SERIES I

The pertinent results of the experiment in Series I are summarized in Tables II–V. Twenty-eight swine fed the severe (TSC) diet received x-irradiation twice. Starting approximately 4 weeks after the last x-irradiation, 22 swine died rather suddenly without apparent signs of sickness. All had advanced coronary atherosclerosis and 24 swine had myocardial infarcts either healed or recent. In 17 out of 24 swine that had myocardial infarcts, there was evidence of congestive heart failure, characterized by marked congestion of the liver, pericardial, pleural or peritoneal effusion.

Among the 14 swine fed the mild (PSC) diet exclusively, only 3 survived for 9 weeks or longer after the first x-irradiation and all 3 had myocardial infarcts. Main coronary arterial walls were markedly thickened in only one swine, but secondary coronary branches were markedly narrowed in all three. No gross visible lesions were found in coronary arteries of four stock-fed swine even though they were x-irradiated twice.

Histologic appearance of coronary lesions varied, depending upon the length of time the swine were on the diet and the length of the period after x-irradiation. The earliest change noted was smooth muscle cells (SMC) proliferation, few cells thick, over normal appearing in-



FIGURE 1.—Gross photograph of anterior view of heart of a swine fed the severe atherogenic diet for 58 weeks and X-irradiated twice. The anterior wall of the left ventricle has been partially cut away to demonstrate the large recent, well-demarcated infarct (arrow). A fibrinous deposit can be seen in patches on the pericardial surface. Segments of the coronary arteries have been previously removed.

ternal elastic membrane. With the progression of the disease the lesions became thicker and cytoplasm of many cells was filled with lipids (Figure 3). Hemorrhage was a prominent and frequent feature of these advanced lesions (Figure 4). Necrosis and calcification also varied from case to case. In some cases, they were quite prominent features (Figure 5).

Complete or virtual occlusion of main trunks or their branches by extensive lesions with or without recent hemorrhage into the plaque were frequently observed in almost all cases. Thrombotic occlusion was not a common feature and was seen in large arteries only in two cases. Occasionally thrombosis of small intramural branches was seen in areas of myocardial infarcts. It is of interest to note that this diet- and x-ray-induced coronary artery disease affects arteries of all calibers (main trunks as well as small branches).

The histologic appearance of the myocardial infarcts was similar to that of human infarcts (Figures 6 and 7).

The four swine that were fed the stock diet and x-irradiated twice and that survived nine

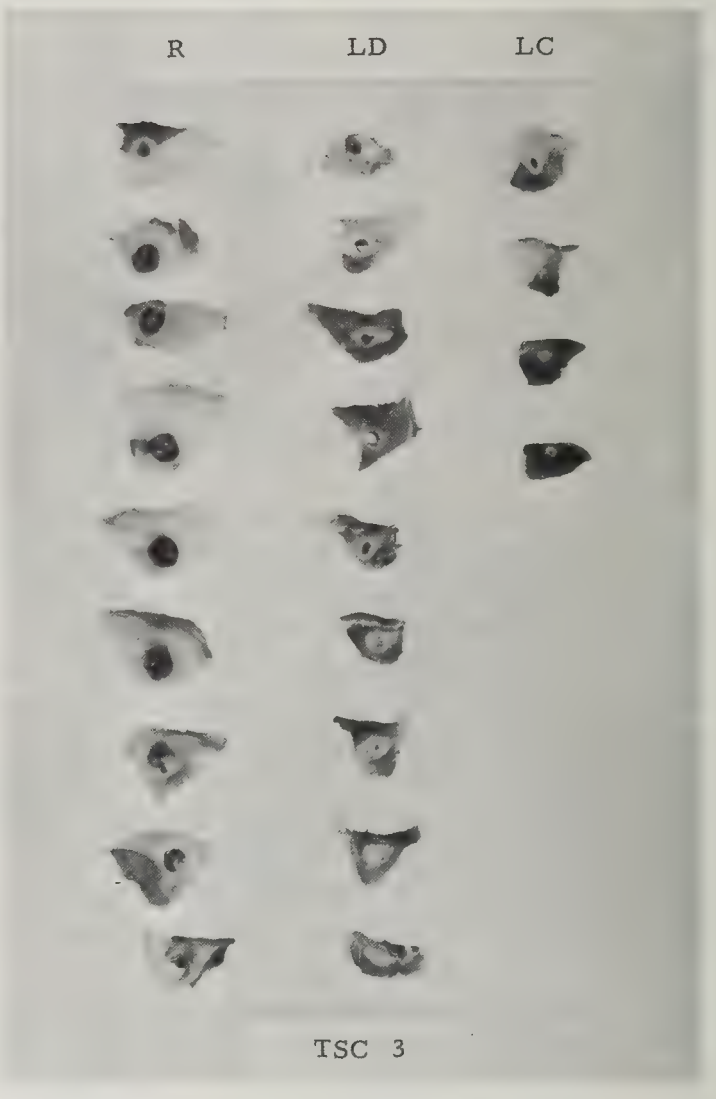


FIGURE 2.—Sequential, alternate segments of the three main coronary arteries of a swine fed the severe diet for 40 weeks and X-irradiated twice (TSC 3 in Table II). The angiograms from this swine, taken prior to sacrifice, are shown in Fig. 9 and can be directly compared to the areas of virtual occlusion seen grossly in these segments. The dark areas of the lesions of the right coronary artery were grossly hemorrhagic.

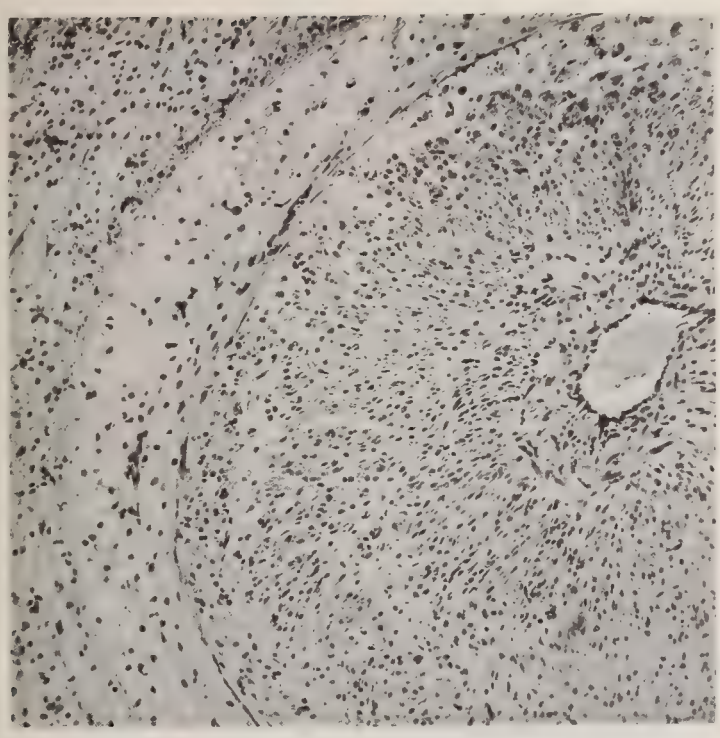


FIGURE 3.—Low power light micrograph of one of the points of near-occlusion of a coronary artery by a proliferative lesion. Foamy, fat-filled cells can be seen, mainly in the media, but most of the lesion is made up of smooth muscle cells. Severe diet-fed, X-irradiated swine.



FIGURE 4.—Very low-power light micrograph of a coronary artery in the epicardial fat. The lesion is similar to that in Fig. 3 but shows, in addition, an area of hemorrhage in the intima and media (dark area at lower right). Severe diet-fed, X-irradiated swine.

weeks or longer after the first x-irradiation showed spindle cell intimal SMC proliferation of one or two cells thick. This degree of cellular proliferation is a common finding even in normal control swine. Acute radiation vasculitis affecting capillaries in rabbit heart as reported by Fajardo and Steward[5] was not seen in our x-irradiated swine.

Electron micrographs of coronary lesions showed extensive proliferation of SMC and a large amount of collagen (Figure 8). Many of the SMC were filled with lipid. These features are also seen in human atherosclerosis but the amount of lipid in SMC is greater in the rapidly produced coronary lesions in the swine.

The coronary angiograms showed extensive luminal narrowing of all coronary arteries (Figure 9A, B, C, D).

In one swine fed the severe diet in Series I, electrocardiographic evidence suggesting myocardial infarction was obtained and this was later confirmed anatomically. Isoelectric or inverted T waves were common in swine even before they were put on experimental diets.

RESULTS—SERIES II

The studies in Series II have not been completed as yet and only the results obtained thus



FIGURE 5.—Very low power light micrograph of a coronary artery with a lesion similar to those in Figs. 3 and 4 but in addition showing extensive calcium deposits in the outer intima and inner media. Severe diet-fed, X-irradiated swine.



FIGURE 6.—Very low power light micrograph of a recent myocardial infarct found in one of the severe diet-fed, X-irradiated swine. Areas contain pale, swollen, partially necrotic muscle cells alternating with areas of marked inflammatory cell infiltrate. A small area of relatively intact myocardium is seen at the far upper right.

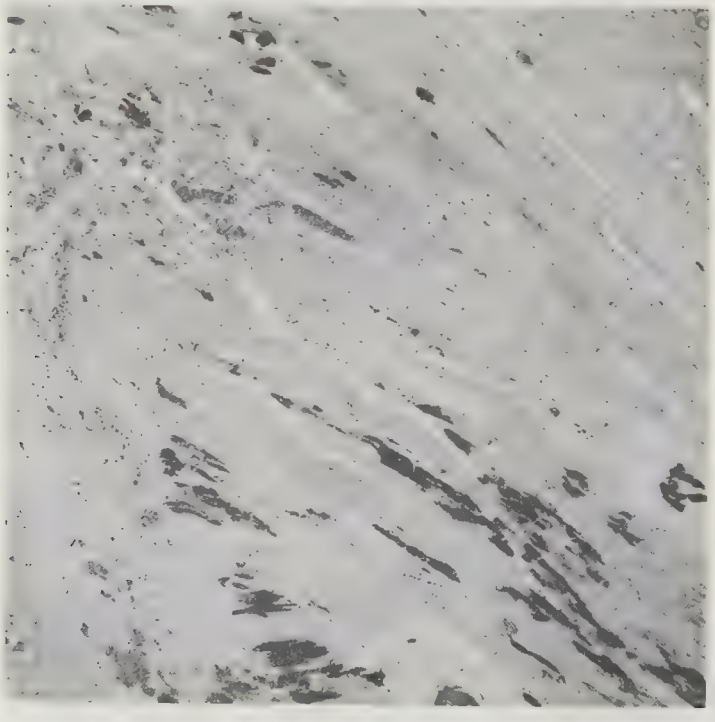


FIGURE 7.—An older, healing myocardial infarct with large, granular dark areas of calcification. Alternating patches of light and dark tissue correspond to the various stages of necrosis proceeding to fibrosis.

far are summarized in this report. Forty swine were fed the moderate (SC) diet which does not contain propylthiouracil (Table I). These swine were x-irradiated twice as in Series I study. This experiment was designed to find out if advanced coronary atherosclerosis and myocardial infarction can be produced in swine without using propylthiouracil. The diet was given in two divided doses in the morning and in the afternoon. The amount of calories given per day was 4,000. Within two months after the swine were put on this diet serum cholesterol levels rose to 300–650 mg%. After 8 weeks on the diet, two doses of x-irradiation were given with 4 week intervals. Thirty-three swine received x-irradiation twice. Starting from approximately 3 weeks after the last x-irradiation,

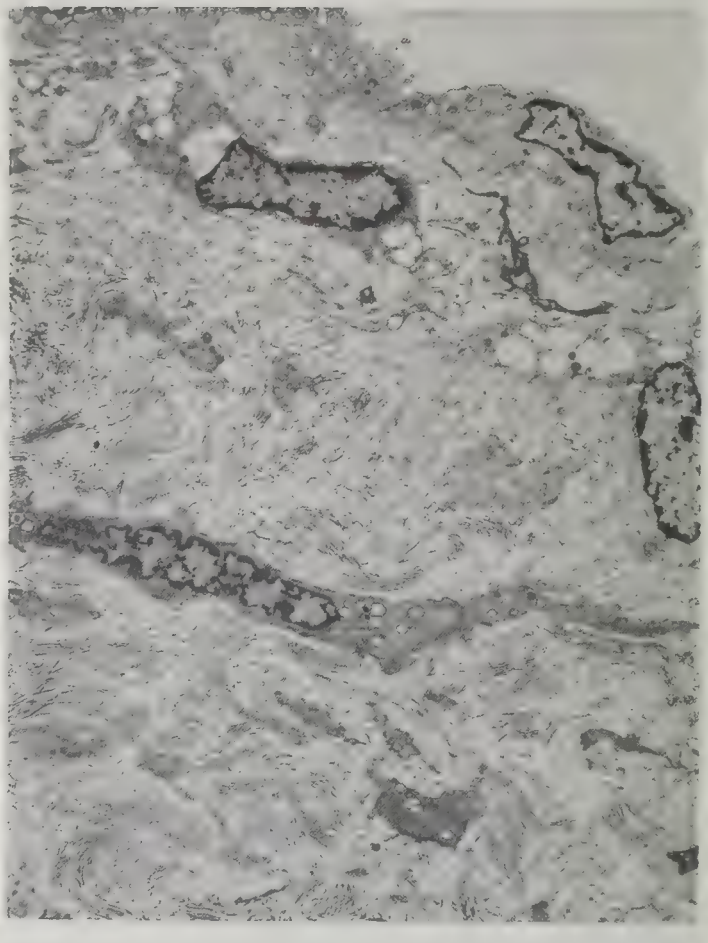


FIGURE 8.—Low power electron micrograph of the endothelial surface and adjacent intima from a large atherosclerotic plaque of a coronary artery in a severe diet-fed, X-irradiated swine. The intimal cells, recognizable as smooth muscle cells, are surrounded by collagen and contain a moderate number of lipid vacuoles.

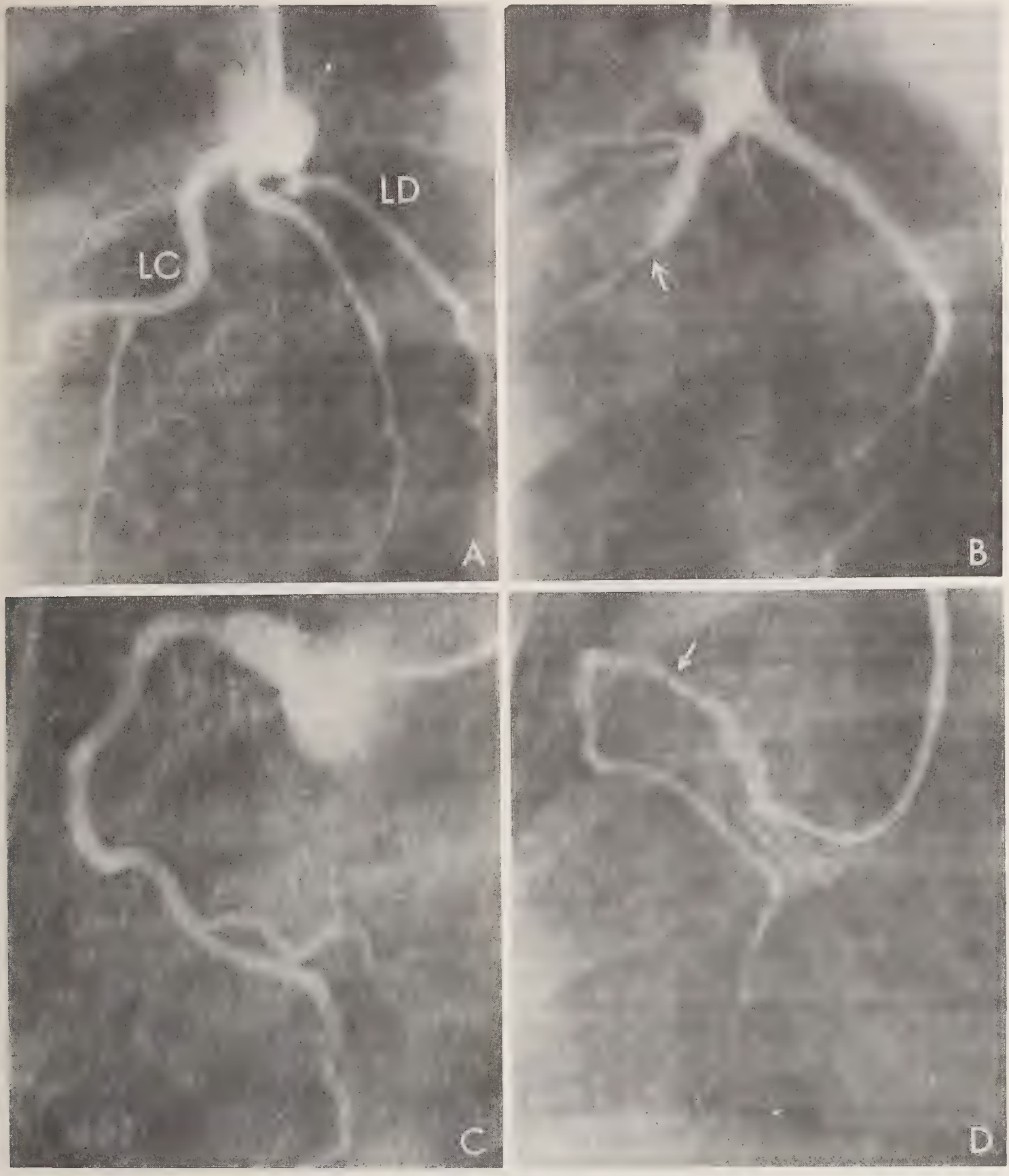


FIGURE 9.—A. Control swine, normal left coronary artery angiogram (LD-left anterior descending; LC-left circumflex). B. Left coronary artery angiogram of swine fed the severe diet and X-irradiated twice shows marked narrowing of left circumflex branch (arrow). C. Control swine, normal right coronary artery angiogram. D. Right coronary artery angiogram of a swine fed the severe diet and X-irradiated twice shows marked narrowing of the lumen by an atherosclerotic plaque.

29 swine died suddenly. All had advanced coronary atherosclerosis and 25 had myocardial infarcts. Most of them were found dead in cages, but five died suddenly soon after experimental procedures, such as coronary angiogram, electrocardiogram or while preparing for anesthesia. In seven swine, it was possible to obtain terminal electrocardiograms. Five showed ventricular fibrillation and two showed ventricular asystole.

Another group of 40 swine were fed the severe (TSC) diet. Dietary regimen and method of x-irradiation were similar to those in the Series I study. The amount of calories given per day was 2,500. Serum cholesterol levels reached 500–1,300 mg% within two months on the severe diet. After 9 weeks on the diet, two doses of x-irradiation were given with 4 week intervals. This group was set up to serve as a control group for the moderate diet group as regards to the degree of coronary atherosclerosis and the incidence of myocardial infarction. Thirty-two swine were x-irradiated twice. Starting from 3 weeks after the second x-ray 28 swine died suddenly. All had severe coronary atherosclerosis and 26 had myocardial infarcts. The majority of them died suddenly in cages without apparent prodromal syndrome, and eleven died suddenly after various experimental procedures as was the case in the moderate diet group. In nine swine terminal electrocardiograms were taken. Six showed ventricular fibrillation and three showed ventricular asystole.

Coronary angiograms were taken in the majority of the swine in Series II. Varying degrees of luminal narrowing of coronary arteries were demonstrated. Although they were considerable individual variations, in general the swine fed the severe diet appear to have more marked narrowing of the lumen than those fed the moderate diet.

Four sets of ECG telemetry systems were used to monitor electrocardiograms of selected swine fed either the severe or moderate diet. In one severe diet-fed swine multiple premature ventricular contractions were detected for 2 days by the telemetry system approximately 6 days before death. This swine died rather suddenly, and ventricular fibrillation was detected in the terminal electrocardiogram (Figure 10).

Terminal electrocardiograms were obtained in 16 swine with sudden death syndrome, of which 9 had been fed the severe diet and 7 had been fed the moderate diet. In 10 swine, terminal ventricular fibrillation was usually preceded by complete AV block, slow idioventricular rhythm or runs of ventricular tachycardia (Figure 11). In six swine, the terminal event was ventricular asystole following complete AV block and extremely slow idioventricular rhythm (Figure 12).

COMMENTS

As presented above, advanced occlusive coronary artery disease with myocardial infarction and sudden death can be induced rapidly in swine by using a combination of injurious and atherogenic agents. The most effective combination, to date, is given in the following sequence: (1) young miniature swine are given a high-fat, high-cholesterol diet with or without propylthiouracil; (2) x-irradiation of the precordium (1,500 r) is given after 8 weeks or more on the diet; (3) a second x-irradiation is given 4 to 5 weeks later. Severe coronary artery disease and its complications begin to appear ap-

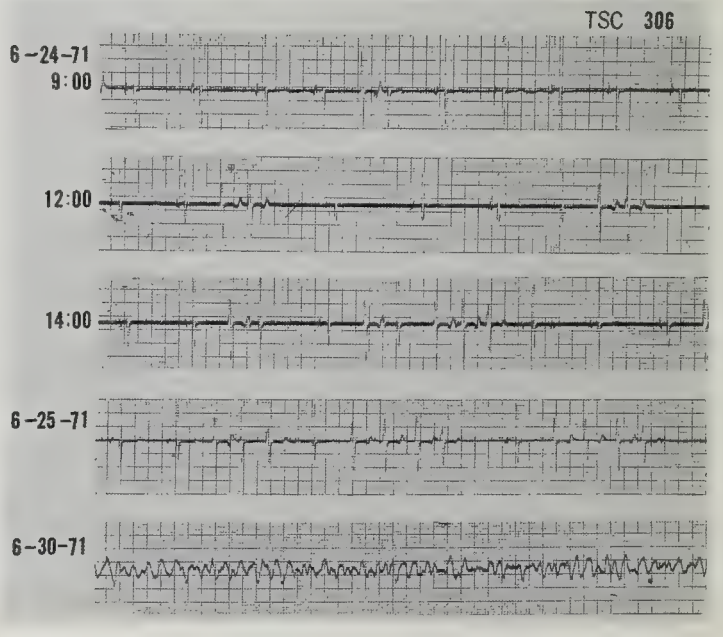


FIGURE 10.—Lead II electrocardiograms of a swine fed the severe diet and X-irradiated twice show increasing number of ventricular premature beats with frequent brief runs of ventricular tachycardia (6/24–6/25) and final ventricular fibrillation (6/30).

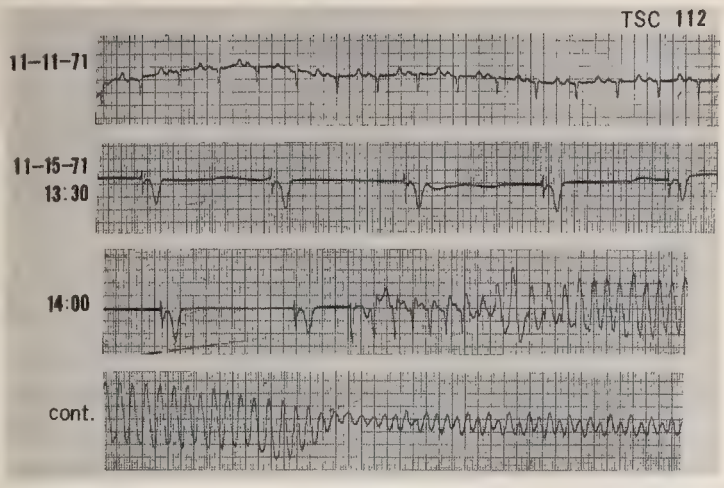


FIGURE 11.—Lead II electrocardiograms of a swine fed the severe diet and X-irradiated twice show sinus rhythm with deep Q wave on 11/11. On 11/15 slow nodal rhythm developed and this was followed by ventricular tachycardia and ventricular fibrillation.

proximately 4 weeks after the last x-irradiation.

The role played by propylthiouracil is not clear. It was used because it is known to produce much higher serum levels of cholesterol than if dietary cholesterol is given without it. It has the inherent disadvantage of inducing metabolic changes that can complicate interpretation of observations related to the ischemic heart disease. However, atherosclerosis of similar magnitude with myocardial infarction and sudden death was produced in swine fed the moderate diet which does not contain propylthiouracil. Thus it appears that propylthiouracil is not an essential feature of the regimen. We have found a remarkable similarity in the

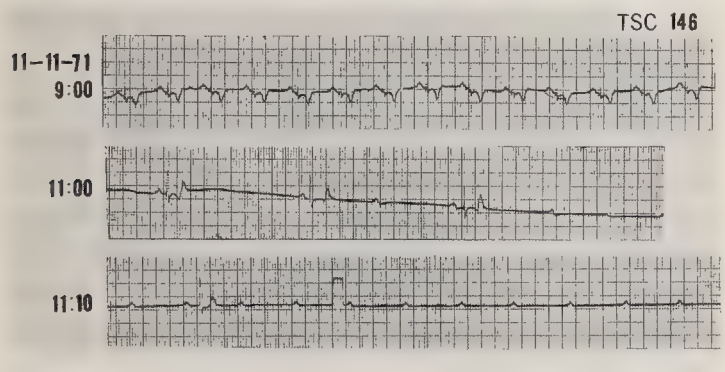


FIGURE 12.—Lead II electrocardiograms of a swine fed the severe diet and X-irradiated twice. On the day of sudden death sinus rhythm was observed initially but it developed advanced AV block which was followed by complete AV block and ventricular asystole.

effectiveness of the moderate diet and the severe diet in producing ischemic heart disease with its complications. The euthyroid state does not appear to protect the swine from the effects of the atherogenic regimen.

The occlusive arterial disease produced in these swine has many similarities to and some differences from that observed in man. The most important feature, from the standpoint of the objectives of the study, is that the lumen is narrowed and blood flow to parts of the myocardium is reduced. The swine frequently develop ischemic heart disease with myocardial infarction, sudden death with cardiac arrhythmia, and occasionally congestive heart failure. Thus the model would appear to be useful for studies aimed at elucidating and preventing physiological derangements resulting from ischemic heart disease.

The lesions involve the major branches of the coronary arteries, with severity varying from branch to branch as in man. However, the marked luminal narrowing extends over longer segments than are usually seen in man. Thrombosis, which is common in man, was not seen grossly even in those with myocardial infarcts. Instead, hemorrhage into plaques was much more frequent than in man.

Histologically all features of the arterial lesions in the swine can be found in man. As in man the predominant cell was the smooth muscle cell, much lipid was present both intra- and extracellularly, and regions of necrosis and calcification could be found. However, the cellular component was more prominent and the necrotic debris and calcification were much less prominent than with advanced coronary atherosclerosis in man. This is to be expected in rapidly developing lesions since the early phase in both man and experimental animals appears to be largely cellular proliferation, with extensive fibrosis and large regions of necrotic debris developing later.

There were no significant gross features other than pericardial fibrosis that could definitely be attributed to x-irradiation per se. The swine treated with x-irradiation alone did not develop demonstrable gross arterial or myocardial lesions. The action of x-ray was apparently through local enhancement of the atherogenic

effect of the diet and drug regimen, but the mechanism by which this enhancement is accomplished is unknown.

We have used the phrase "sudden death" to refer to any death in which preceding signs of severe illness were not recognized in the swine. Since most of those that died suddenly had one or more myocardial infarcts, it is obvious that they did actually have severe preceding disease. On 16 swine that died suddenly terminal electrocardiograms were obtained; 10 showed ventricular fibrillation and 6 showed ventricular asystole. In man with coronary heart disease dying suddenly, it is usually speculated that they died of ventricular fibrillation. Cobb et al.[6] reported recently that ventricular fibrillation was the main cardiac arrhythmia among prehospital emergency cardiac cases treated by their mobile intensive/coronary care unit. Since the immediate cause of death in those 16 swine dying suddenly was proved to be either ventricular fibrillation or ventricular asystole, it is reasonable to assume that many or most of the swine that died suddenly in our previous experiments had cardiac arrhythmias.

SUMMARY

The swine model developed in the current study should be of considerable value in studies of sudden death and also for the scientific trial of therapeutic devices and surgical procedures prior to their use in man.

REFERENCES

1. Thomas, W. A., Florentin, R. A., Nam, S. C., Kim, D. N., Jones, R. M., and Lee, K. T. Pre-proliferative phase of atherosclerosis in swine fed cholesterol. Arch. Path. 86:621–643, 1968.
2. Florentin, R. A., and Nam, S. C. Dietary-induced atherosclerosis in miniature swine. I. Time of development and morphologic characteristics of lesions. Exp. Mol. Pathol. 8:263–301, 1968.
3. Lee, K. T., Jarmolych, J., Kim, D. N., Grant, C., Krasney, J. A., Thomas, W. A., and Bruno, A. M. Production of advanced coronary atherosclerosis, myocardial infarction and "sudden death" in swine. Exp. Mol. Pathol. 15:170–190, 1971.
4. Daoud, A. S., Jarmolych, J., Zumbo, O., Fani, K., and Florentin, R. Pre-atheroma phase of coronary atherosclerosis in man. Exp. Mol. Pathol. 3:475–484, 1964.
5. Fajardo, L. F., and Steward, J. R. Experimental radiation-induced heart disease. Amer. J. Pathol. 59:299–315, 1970.
6. Cobb, L. A., Conn, R. D., and Samson, W. E. Prehospital coronary care: The role of a rapid response mobile intensive/coronary care system. Circulation 44 (Suppl. II):45, 1971.

DISCUSSION

Chairman Kezdi: Dr. Lee, your coronary angiograms look frighteningly similar to human coronary angiograms. I just would like to ask you one question. When you irradiated the animals, you irradiated the chest. Do you believe that the same thing would occur if you irradiated the abdominal area?

Dr. Lee: Yes, we like to think so. In other studies, we are now irradiating the carotid arteries. We have preliminary data indicating that this produces quite an advanced lesion. However, we have not done the abdominal aorta; but I have every reason to believe that the result should be the same.

Thomas C. Militano, NIH, Bethesda, Md.: In our previous discussions, Dr. Lee, you indicated to me that you felt that the role of the X-ray was merely to produce intimal damage, so that it accelerated the effect of the cholesterol, and you indicated that there were other techniques that you were considering to produce intimal damage. One of those you mentioned interested me because it was a generalized technique, a serum sickness type model. I wonder if you could comment on that?

Dr. Lee: Yes, we talked previously about X-ray injury. We like to think that X-ray causes ionizing damage to the endothelial cells or cellular wall and the latter increases the rate of filtration of lipids into the intimal cells and this in turn accelerates the development of the lesion. In regard to other methods of producing atherosclerotic lesions, we have previously demonstrated that an injury more comparable to a naturally-occurring injury, experimental serum sickness, in combination with a hypercholesterolemic diet, resulted in enhancement of atherosclerotic lesions in aortas of rabbits. However, we have not yet tried this technique in swine.

H. L. Brooks, University of Chicago: Dr. Lee, do you believe that you could find some way

in this fine model to determine the exact point just before a pig is going to have an infarct so that you could study the effects of ischemia? Do you have, for example, some set protocol whenever you do coronary angios every set period of time, or say a stress test of this type?

DR. LEE: The answer is we cannot predict when a particular pig is going to drop dead. Quite a number of our final electrocardiograms were taken before or during a procedure involving strain such as when we pull the pigs out of the cage for anesthesia for the angiograms and at that time some of the pigs develop ventricular fibrillation. But it is difficult to determine by looking at the pig whether he is going to die suddenly. We lose pigs sometimes while doing angiograms as a result of lethal arrhythmia's which we have not been able to prevent entirely. We sometimes use anti-arrhythmic drugs, but their effectiveness is questionable.

CHAIRMAN: Dr. Lee, what is the actual time sequence of your experimental protocol? Do you irradiate and then start feeding, and would you assume that if you started feeding, say for eight months, and if you then irradiated, would the time needed for producing the occlusion be decreased or remain unchanged?

DR. LEE: I failed to mention my time schedule for feeding and X-ray. When we get the pigs, we condition them until they reach about 10 kilograms in weight. Then they are fed an atherogenic diet for approximately two months in order to increase their cholesterol levels to those levels that I showed you earlier. At the end of this time the first x-irradiation is given and this is followed by the second x-irradiation in one month. Approximately 2 months after the last x-irradiation the pigs begin to die suddenly. The entire experimental procedure takes about five to six months.

D. M. KRAMSCH, Boston University Medical Center, University Hospital, Boston, Mass.: Dr. Lee, we know from your previous work that you could use irradiation without uracil, bile acids, and cholesterol, which will cause quite severe coronary artery lesions in pigs, yet no myocardial infarction. Now this makes one wonder whether true X-ray irradiations damage only the arteries or the myocardial muscle cells as well? You showed lesions in the intimal small vessels; the same agent must have gone through the myocardial muscle cells. My question is, did you perhaps study, by electron microscopy or biochemically, any irreversible damage in the myocardial muscle cells? What happened to the CBK enzyme system?

DR. LEE: Yes, we produced quite a severe lesion, previously without x-irradiation as I mentioned in my introduction, but none was followed by myocardial infarction. We like to believe that with X-ray and with this regimen, the lesion is more severe, and also, in this way, possibly not only large vessels but also smaller vessels are involved, as I demonstrated in the picture. Accordingly, this would increase the chances of an infarct.

Myocardial biochemical studies are done in our laboratory at present. We are finding some decreased production of ATP and increased oxygen uptake on these myocardiums. But whether or that has a direct impact on infarction, I do not know.

EXPERIMENTAL MYOCARDIAL INFARCTION IN NONHUMAN PRIMATES

M. R. Malinow, J. D. Hill, M. Lees, W. P. McNulty and A. J. Ochsner, III*

Myocardial infarction was attempted in 60 "chair"-restrained, nonanesthetized *Macaca mulatta* by the tightening of a snare previously placed around the left anterior descending coronary artery. Aortic flow, ECG, arterial and left atrial pressures were monitored. Postmortem examinations showed extensive anterolateral septal infarction in animals surviving more than 12 hours. In 26 monkeys, a discriminant analysis, based on hemodynamic data obtained within 30 minutes after infarction, resulted in a retrospective prediction that was accurate in all animals dying from cardiogenic shock and in 93% of the surviving animals. In 13 monkeys, nuclide-labeled microspheres (Scandium-46, Strontium-85 and Iodine-125) were injected through a catheter into the left atrium; organ blood flows were determined before and 15 and 45 minutes after ligation of the coronary artery. The results demonstrated a reduction of blood flow to all tissues, but the differences were not significant in the liver and the adrenals. A redistribution of the decreased cardiac output favored the brain, heart, liver and adrenals where the reduction was less than in the rest of the body.

INTRODUCTION**

Although atherosclerosis occurs frequently among monkeys,[1,2] myocardial infarction is extremely rare. The latter has been reported in single cases of *Lemur macaco*,[3] *Cercocebus collaris*,[4] and *Hylobates concolor*[5] by Hamerton, and in single cases of chimpanzees reported separately by Manning,[6] Burlingame,[7] and Ratcliffe;[8] Ratcliffe also described a transmural infarction in a *Gorilla gorilla*.[9] Myocardial infarction was observed by Taylor et al.[10] in one of the 15 rhesus monkeys fed a high fat-high cholesterol diet for 40 months and by Andrus et al. in 3 of 14 chimpanzees that had received a high fat-high cholesterol diet for 12 months.[11] Hemodynamic or electrocardiographic studies have not been reported in any of these animals.

The coronary arteries of monkeys were ligated by earlier investigators,[12,13] and in some cases the ensuing myocardial infarction was studied. Extensive research in this field has been conducted by deWaart et al.,[14] who ligated either the anterior descending or the right coronary artery in 31 crab-eating macaques *(M. irus);* in one of these animals, both coronaries were ligated. Three-lead electrocardiograms were obtained and some of the animals were followed up to 6 months. Although, in general, the earliest changes could not be documented because records were obtained after the chest had been closed, the tracings were well correlated with the location of the infarction, and the electrocardiographic evolution was carefully studied. Myocardial infarction has also been induced in nonrestrained *M. mulatta* by radio control of a snare placed around the left anterior descending coronary artery and brought through the anterior chest by means of a polyvinyl tubing.[15] After the animals had recuperated (generally a week), the snare was secured to the capstan of a motor with an attached battery-operated electronic switch. Electrocardiographic leads (CF_1) were sutured and connected to a miniaturized amplifier and transmitter. When the animals had been returned to their cages, the motor was remotely activated with a transmitter and the coronary snare was thereby tightened. The ECG was monitored on an oscilloscope and recorded at appropriate intervals. Tightening of the snare was stopped when signs of electrical injury appeared. One of the 6 monkeys died of ventricular fibrillation; the rest were sacrificed 3 to 4 weeks after occlusion. The electrocardiographic pattern was similar to that in human cases of anterior wall infarction, and necrosis of the myocardium was found in the postmortem studies.

* Oregon Regional Primate Research Center, Beaverton, Oregon and University of Oregon Medical School, Portland, Oregon

** Supported by Contract PH-43-68-686/The Artificial Heart-Myocardial Infarction Program of the National Heart and Lung Institute and Grants FR 00163 and HE 12594 from the National Institutes of Health.

A myocardial infarction-like syndrome has been induced in "chair"-restrained *M. mulatta* by isoproterenol given subcutaneously.[16] Although the effective dose was extremely high (100 mg/kg), only 2 of 16 monkeys died from its administration. The electrocardiogram obtained under these extreme conditions was similar to the one observed in myocardial infarction in man: abnormal Q waves, elevated S-T segment, and T-wave inversion. However, unlike the results in man, the Q waves disappeared after 8 to 10 days and the ECG's were practically normal after 20 to 30 days. The hemodynamic changes, which tended to disappear in a few days, included a lowering of the blood pressure, which, however, remained above shock levels, peripheral vasodilation, tachycardia, and a moderate early increase in cardiac output (dye injection method). SGOT became elevated 12 to 24 hours after the injection of isoproterenol and returned to normal 3 to 4 days later. Strangely enough the microscopic changes, extreme nuclear and fibrillar disruption, were apparently reversible up to 60 days since no granulation tissue had replaced the muscular fibers.

Recently we reported the hemodynamic and pathologic changes observed in "chair"-restrained *M. mulatta* when myocardial infarction was induced by the tightening of a snare that had been placed around the left anterior descending coronary artery a few days previously.[17] This paper as well as some unpublished observations[18] are freely quoted here.

MATERIAL AND METHODS

Sixty adult male and female *Macaca mulatta* weighing between4.5 and 9.5 kg were studied. Nine animals used during the development of the technique are not reported here. Twenty mg of succinylcholine chloride (Quelicin,(R) Abbott Laboratories) were administered intravenously and a cuffed endotracheal tube was inserted. General anesthesia (0.5 to 1.25% halothane (Fluothane, Ayerst Laboratories, Inc.) vaporized in a Fluotec vaporizer with 40% nitrous oxide and 60% oxygen) was administered with a Boyle's anesthesia machine.

A polyvinyl chloride catheter placed in the aorta via the femoral artery was connected to a transducer (Statham Laboratories, Inc.) to monitor blood pressure during surgery and again several days later during the ligation experiment. The pericardium was opened after a midline sternotomy, and an electromagnetic square wave flow probe (Biotronex Laboratories) was placed around the aorta just superior to the coronary arteries. A polyvinyl catheter was placed in the left atrium by means of a purse-string suture. A nylon filament snare (8 lb. test), loosely placed around the left anterior descending coronary artery 2 to 3 mm distal to the origin of the circumflex branch, was introduced into a polyvinyl tube with a flared tip and the tube was passed through an intercostal space; the flared end was sutured to the pericardium and the tube was filled with vaseline to form an air seal. A slow continuous intravenous drip of 5% dextrose in water was maintained and, depending upon blood loss, whole blood from donor rhesus monkeys was transfused. If the blood pressure dropped below normal levels mephentermine sulfate (7 mg, Wyamine, Wyeth Laboratories, Inc.) was given intravenously. The pericardium and thorax were closed routinely after the implanted probe wires and left atrial catheter were brought through the chest. After surgery and intramuscular injections of penicillin and procaine penicillin G (Bicillin CR, 1,200,000 units), the monkeys were placed in specially designed suits and recuperated in a cage for 3 to 5 days.

Series I

Twelve male and 22 female adult *M. mulatta* were considered suitable for this study, the experimental design of which is shown in Figure 1. On the morning of occlusion, subcutaneous wires were sutured to the proximal part of the four limbs and to three chest positions (V_1 and V_3 were 1 cm below the right and left mammary papilla, respectively; V_5 was placed 1 cm lower than V_3 and at the anterior axillary line). The animals were placed in restraining "chairs" and pressure transducers were located at the level of the heart. Four to 6 recordings at 15-minute intervals were averaged for the control values. After an apparent steady state had been reached, the snare was gradually tightened

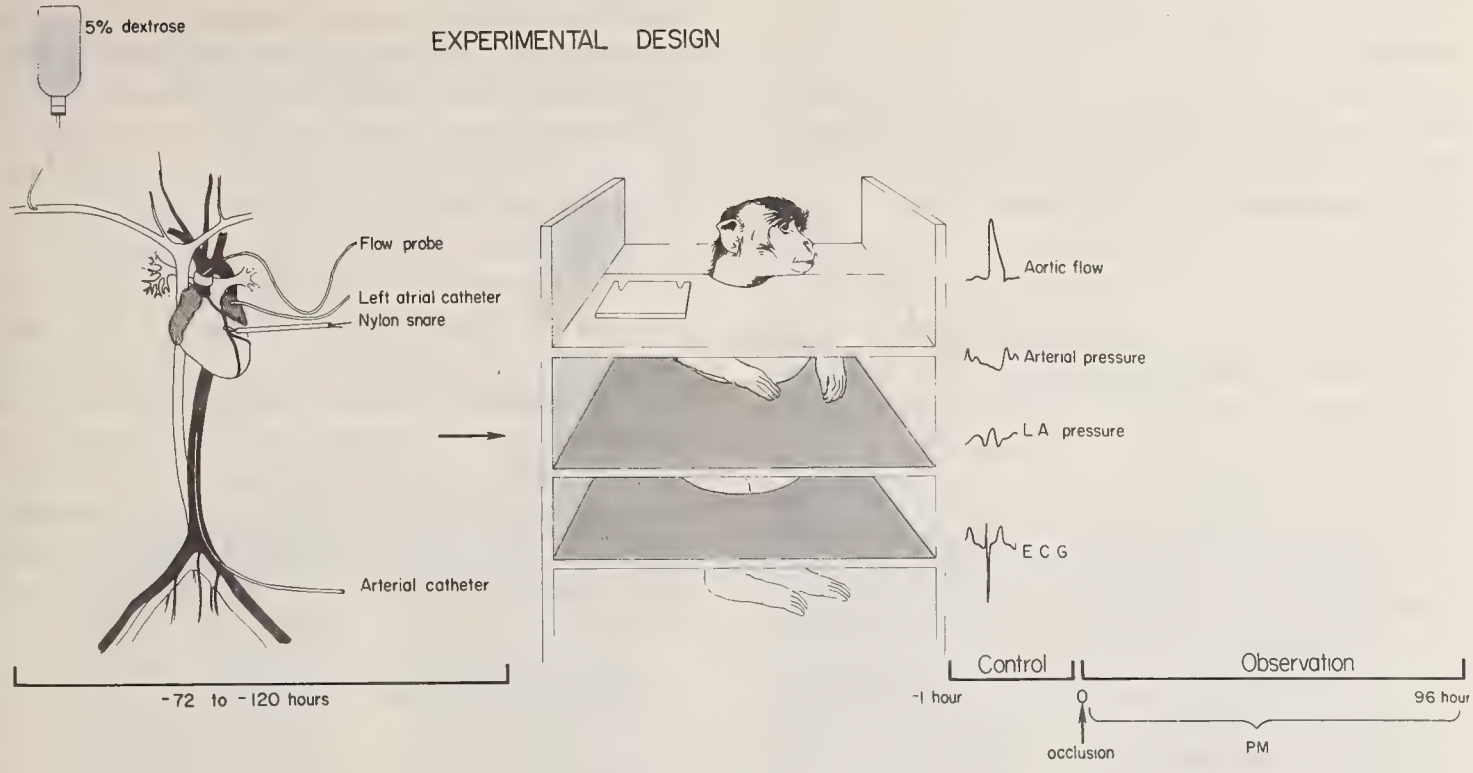


FIGURE 1.—Experimental design (discussed in the text).

for 1 to 2 minutes until an injury current was observed in any of the precordial leads in the ECG constantly monitored on a 6-channel oscilloscopic recorder (Sanborn, Model 350). The following parameters were recorded: (1) 9 leads ECG; (2) blood flow in the ascending aorta (considered to represent cardiac output when coronary flow is disregarded); (3) left atrial pressure; and (4) aortic systolic, diastolic, and mean pressure. After infarction, recordings were taken at 5-minute intervals for ½ hour, 10-minute intervals for 1 hour, 30-minute intervals for 2½ hours, and 1-hour intervals thereafter until 5:00 p.m., and significant changes were recorded. External defibrillation with D.C. current countershock (American Optical Co.) at 200 watt seconds was applied if serious ventricular arrhythmias (ventricular tachycardia, flutter, or fibrillation) occurred. The animals were periodically observed until midnight, but no recordings or attempted defibrillations were made after 5:00 p.m. Recordings were then made twice daily until the experiment was terminated.

For retrospective prediction, we used discriminant analysis[19] based on the general formula: $L = K + aW + bX + cY + dZ$
where:

L = discriminant function
K = constant
a = coefficient for aortic systolic blood pressure W
b = coefficient for aortic diastolic blood pressure X
c = coefficient for aortic mean blood pressure Y
d = coefficient for aortic mean blood flow Z

At selected periods after ligation, values were obtained for systolic, diastolic, and mean blood pressure and aortic flow. With these data and a program written in Fortran II and executed on a Xerox Data System Model 920 digital computer, the constant and coefficients were determined for the "Alive" (L_A) and "Dead" (L_D) groups separately (see results). The pressure and aortic flow from individual animals were then used with the previously generated constant and coefficients from each group. This produced an L_1 and L_2 function for every animal. Monkeys whose L_1 was larger than the L_2 were predicted to survive, and the retrospective

predictions were compared with the observed events.

Complete postmortem examination was performed on all animals that had died during the observation and on those killed 24 to 96 hours after the tightening of the snare.

Series II

In 17 animals, 9 male and 8 female adult *M. mulatta,* weighing between 5.1 and 8.3 kg, nuclide-labeled microspheres (Scandium-46, Strontium-85 or Iodine-125) about 50 μ in diameter (Minnesota Mining and Manufacturing Company) were injected into the left atrium during the control period and repeated 15 and 45 minutes after the induction of myocardial infarction. About 100 μCi, corresponding to 300,000 microspheres of each isotope, were injected per animal. Cardiac output was measured by indocyanine green dye dilution curves (mean of 2 or 3 curves) before each nuclide injection. The dye was injected via the left atrial catheter, and blood was withdrawn from the aortic catheter by a pump (Model 600-900, Harvard Apparatus Co., Inc.) at 9.89 cc/min through a cuvette densitometer (Model 1031R, Gilford Instrument Co.) and recorded on a polygraph (Model M5P, Gilson Medical Electronics). Arterial blood samples were taken for measuring pH and oxygen and carbon dioxide tension with a pH meter and gas monitor (Radiometer, Copenhagen) before and after the induction of myocardial infarction. After the animal's death, the body was dissected into its various organs. The heart was examined grossly and the location of the snare was checked; a complete autopsy was not done since it would interfere with the radioisotope analysis. Larger parts, e.g., carcass and skin, were incinerated, and an aliquot of about 10 percent of the ash was taken for radioactivity measurement. The validity of this method had been previously confirmed by total analysis of the ash from other animals. Large organs (e.g., kidneys and liver) were incinerated and totally analyzed for radioactivity. Small organs (e.g., adrenal glands and pituitary glands) were placed directly into weighed sample tubes for analysis of radioactivity. Thus, the total radioactivity of each radionuclide in the monkey was measured. The proportion of radioactivity in an organ multiplied by the cardiac output was then a measure of the organ blood flow:

$$\text{Organ blood flow (ml/min)} = \text{Cardiac output (ml/min)} \times \frac{\text{organ radioactivity (CPM)}}{\text{total CPM in animal}}$$

The radioactivity from each separate nuclide in individual sample tubes was determined in a three-channel Packard gamma spectrometer, Series 5000.[20] Organ blood flow data were expressed as percentages of systemic flow perfusing each organ and flow per 100 gm. of tissue.

RESULTS

Series I

Five animals died postoperatively before ligation. In the 29 survivors the snare was ligated; in 3 of these the ligation was unsuccessful because misplacement of the snare excluded the coronary artery or included part of the pulmonary valve, or the snare broke. The remaining 26 were divided into two groups: (1) Group I ("Dead") included the 11 monkeys that died within 6½ hours from myocardial infarction with a syndrome resembling cardiogenic shock; (2) Group II ("Alive"), the 15 monkeys that survived myocardial infarction more than 12 hours and in many cases were terminated 24 to 96 hours after infarction. No deaths occurred between 6½ and 12 hours after infarction. Five animals that died during the first night but more than 12 hours after infarction may have had cardiogenic shock; however, the possibility of a fatal arrhythmia cannot be ruled out. Since their aortic blood flow was never under 55% (percentages refer to control values taken as 100%) and mean aortic pressure was never less than 70% during the observed period, they were placed in Group II ("Alive").

Immediately after the snare was pulled, the animals became agitated for 1 to 2 minutes. In general, animals with initial decreases of more than 50% in aortic blood flow and 30% in mean aortic pressure appeared dazed, did not respond

immediately to external stimuli, did not eat or drink, did not survive longer than 6½ hours after myocardial infarction, and presumably died from cardiogenic shock. Animals that survived more than 12 hours remained alert and responsive to their environment and ate and drank normally.

Electrocardiogram. Two main patterns were observed. (a) In 25 animals, the S-T segment became slightly elevated in leads I and aVL and the precordial leads and depressed in leads II, III, and aVF; the S-T displacement became marked 3 to 4 minutes later (Figure 2). About 30 minutes after ligation, a small Q wave appeared in the standard leads and in leads aVF and V_1 and increased in amplitude afterwards; a QS pattern developed in the precordial leads. Twenty-four hours later the S-T displacement tended to disappear in the frontal plane but remained elevated in the precordial leads. In the survivors, the QS pattern persisted up to 4 days when they were killed. Postmortem examina-

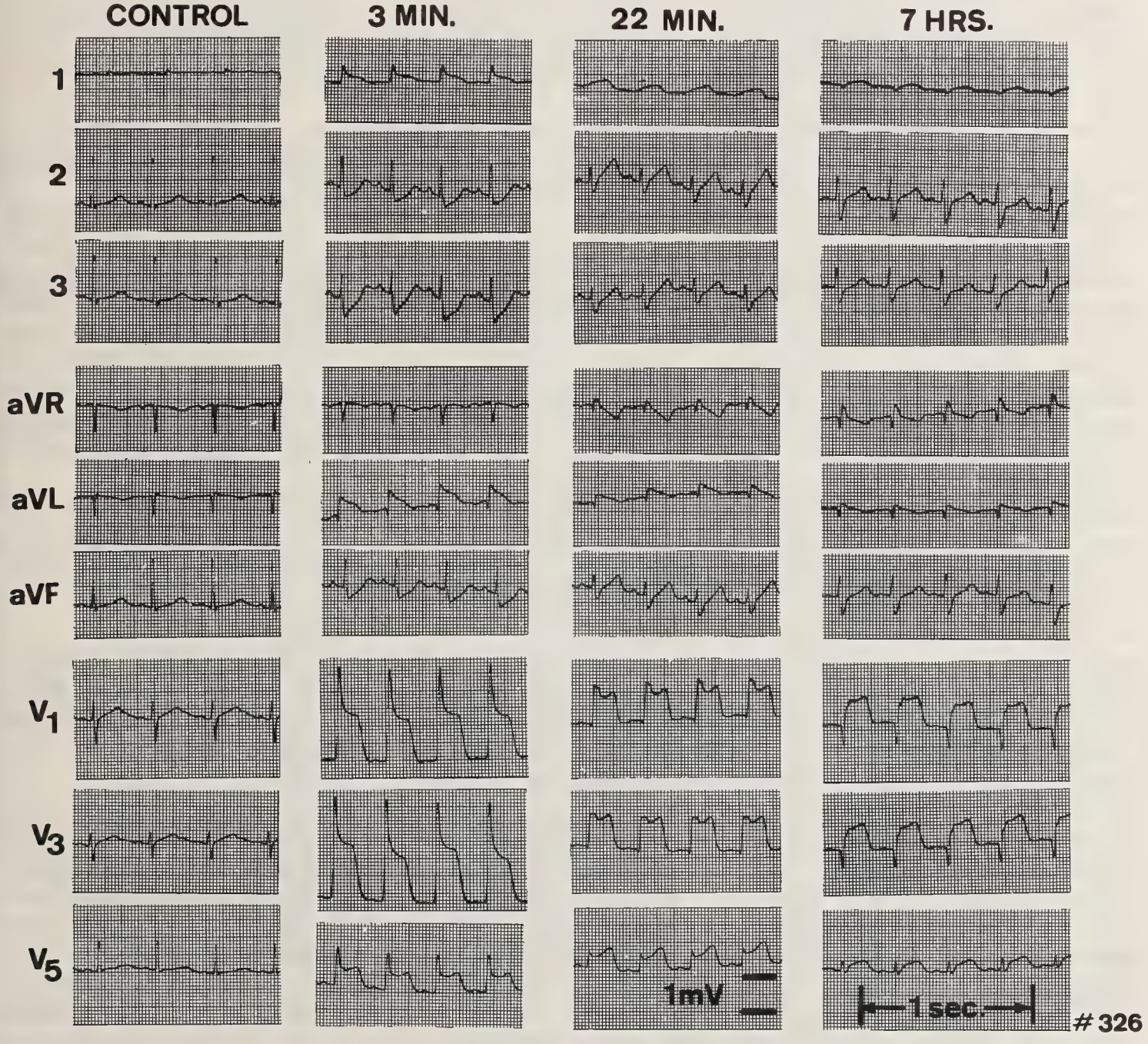


FIGURE 2.—Electrocardiographic changes in a monkey after ligation of the anterior descending coronary artery. Pattern (a).

tion showed that this pattern corresponds to infarction of the anterior, lateral, and septal part of the left ventricle as well as of the posterior part of the apex and a small part of the right ventricular wall. (b) Only one animal differed from pattern (a) although the ECG changes had a similar time sequence (see Figure 3) : the S-T segment was elevated in leads I, II, aVL, V_3, and V_5 and depressed in leads III, aVR, and V_1. A Q wave was observed in leads I and V_5, whereas the amplitude of the R wave decreased in V_1 and V_3. This pattern, corresponding to an infarction of the lateral aspect of the free wall of the left ventricle, was confirmed at autopsy. At 3 days an inverted T wave was observed in leads II, III, and aVF; however, since it was also seen in the control tracing, it may be unrelated to ischemia and may represent an abnormal repolarization pattern sometimes seen in a small number of intact rhesus monkeys.[21]

Right bundle branch block (RBBB) was observed in 13 animals after snare ligation. Apparently the conduction defect did not affect survival and occurred about equally in both the "Alive" and "Dead" groups. In most cases, it was evident in the one-hour recording after infarction; however, a few animals did not develop RBBB for several hours.

Premature ventricular beats were common immediately after coronary occlusion, but the most serious and frequent arrhythmia was ventricular fibrillation, 1 to 9 episodes of which occurred in 18 animals. Ventricular fibrillation was noted in 2 animals about 24 hours after ligation; the other 57 episodes were observed within 40 minutes after coronary occlusion. These arrhythmias were successfully terminated with D.C. countershocks. Ventricular fibrillation did not appear to affect survival; 35 episodes occurred in 11 of the 15 (73%) surviving monkeys and 24 episodes in 7 of the 11 (63%) animals of Group I.

Hemodynamics. The hemodynamic changes were more severe in the animals in Group I than in Group II (Figure 4). The aortic flow dropped to 45% of the control values shortly after coronary ligation and did not rise above 50% of the control values throughout the survival period. The aortic mean pressure fell to about 60% of the control values, rose in some cases to within 75% of the control values about one hour after ligation, and then progressively declined until death. In Group II animals, the aortic flow dropped to about 65% of the control values immediately after coronary ligation but generally never below 55% at any time during the experiment. The aortic mean blood pressure fell initially to about 75% of the control values; afterwards, it rose earlier and more rapidly than in Group I animals to about 80 to 85% of the control values and remained relatively stable thereafter.

The values of pulse pressure, left atrial pressure, and heart rate considerably overlapped in Groups I and II animals with no significant differences between the two. Of interest was the heart rate observed in the "control" period, 214 $\pm$ 15 and 211 $\pm$ 14 (mean $\pm$ S.D.) in monkeys of Groups I and II, respectively.

Systolic, diastolic, and mean blood pressure and aortic flow were utilized in the discriminant analysis. Ten data arrangements were analyzed (Table I). In data arrangement #1, all values were selected at the time of the lowest diastolic blood pressure recording during the 30-minute post-ligation period with blood pressure values expressed in mm. Hg. The function obtained for the "Alive" and "Dead" groups was, respectively:

$$L_A = -21.400 + .296W + .44X - .302Y + .320Z$$

$$L_D = -10.156 + .233W + .128X - .178Y + .186Z$$

(see Methods for symbols).

Data arrangement #1 correctly predicted 100% (11 out of 11) of the monkeys dying from myocardial infarction with shock and 93% (14 out of 15) of the monkeys surviving more than 12 hours.

Pathology. A large myocardial infarct was found in every animal that survived at least 12 hours after ligation. The distribution of necrosis was fairly constant; nearly all of the apex and higher sections up to the level of the ligation in the anterior and the lateral walls of the left ventricle, the anterior half to two-thirds of the septum, and a small part of the anterior right ventricular wall. Generally a narrow zone of subendocardial muscle remained viable. However, in one case the necrosis extended to the

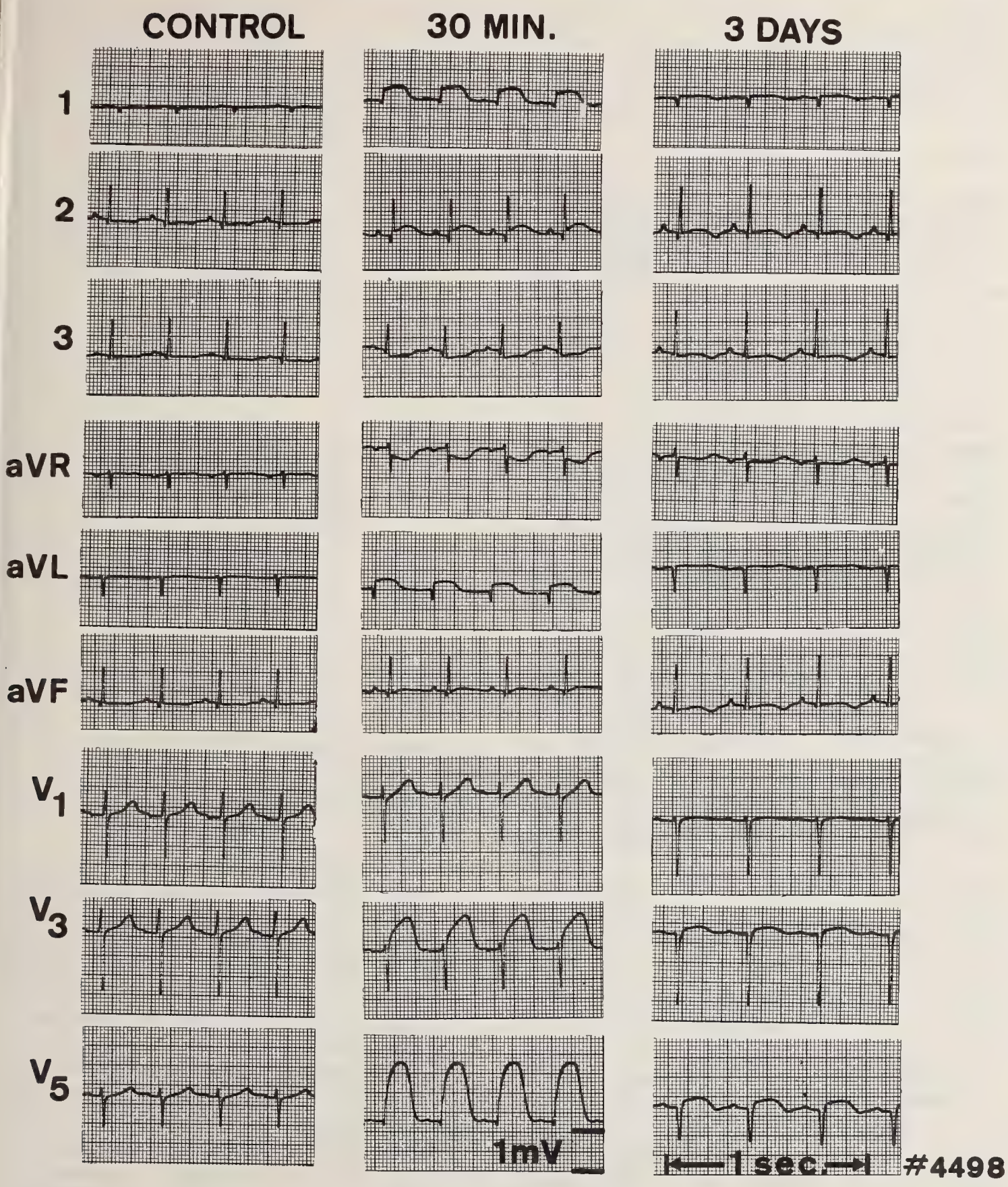


FIGURE 3.—Electrocardiographic changes in a monkey after ligation of the anterior descending coronary artery. Pattern (b).

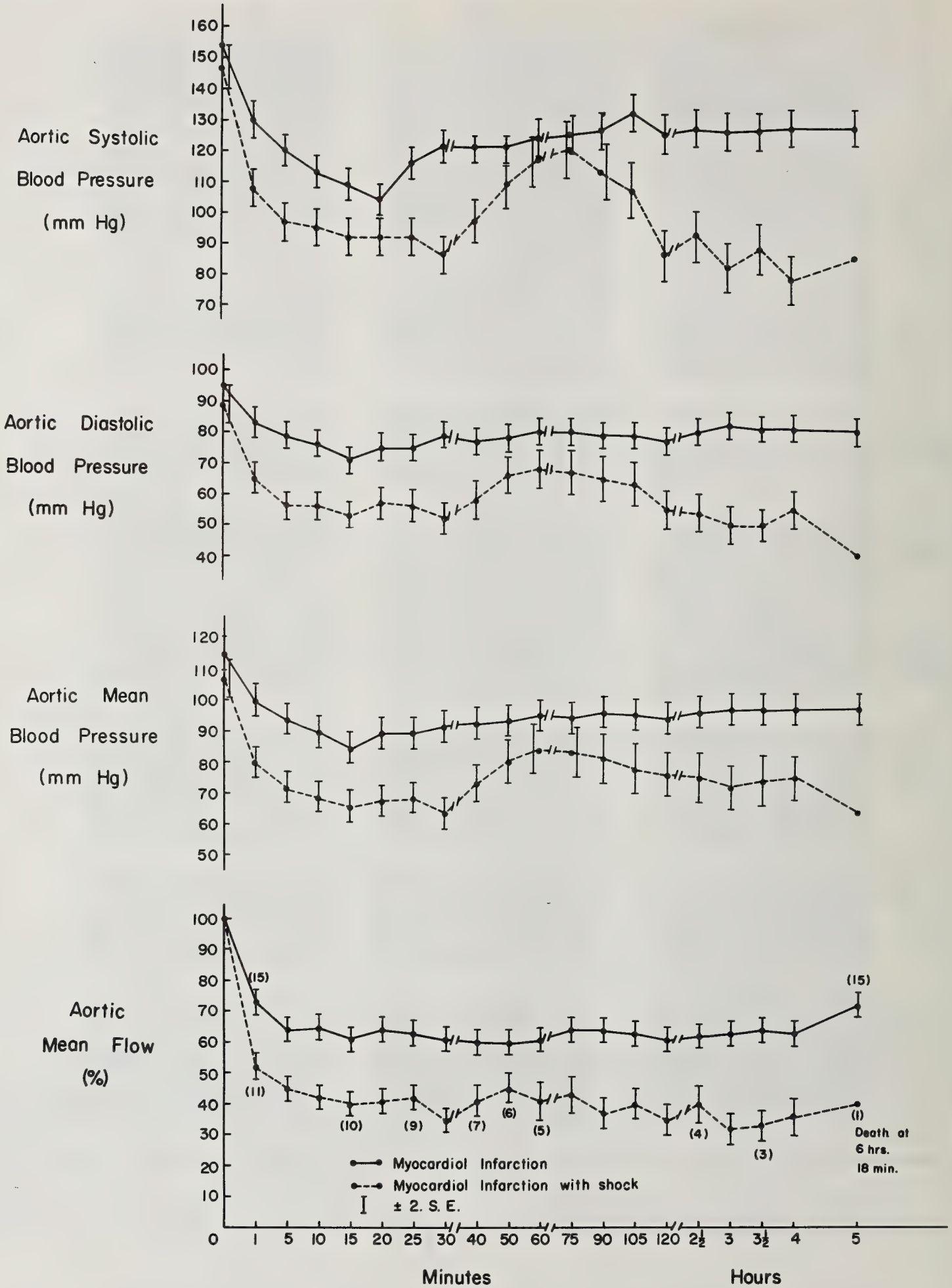


FIGURE 4.—Hemodynamic changes in monkeys after ligation of the anterior descending coronary artery. Figures refer to number of animals (Series I).

TABLE I.—*Retrospective Prediction by Discriminant Analysis of "Alive" and "Dead" Monkeys After Experimental Occlusion of the Left Anterior Descending Coronary Artery*

Data Arrangements	Percent Correctly Predicted* Group I	Group II
1. All values selected at the time of lowest diastolic blood pressure recording during the 30-minute postligation period. Systolic, diastolic, and mean blood pressure in mm.Hg. and aortic flow in percentage of control value.	100	93
2. Same as #1 but all values in percentage of their control values.	91	80
3. Same as #1 but without aortic flow.	82	80
4. Same as #2 but without aortic flow.	73	87
5. All values selected at the time of the lowest aortic flow recording during the 30-minute postligation period. Systolic, diastolic, and mean blood pressure in mm.Hg. and aortic flow in percentage of control values.	80	92
6. Same as #5 but all values in percentage of their control values.	90	92
7. All values selected at the time of the time the lowest systolic blood pressure recording during the 30-minute postligation period. Systolic, diastolic, and mean blood pressure in mm.Hg. and aortic flow in percentage of control value.	82	93
8. Same as #7 but all values in percentage of their control values.	100	87
9. Same as #7 but without aortic flow.	82	80
10. Same as #8 but without aortic flow.	64	80

* Group I ("Dead")—Eleven monkeys which died within 6½ hours from myocardial infarction with shock. Group II ("Alive")—Fifteen monkeys which survived myocardial infarction more than 12 hours.

ventricular lumen, and a mural thrombus was attached; thrombi were found in the renal arteries, presumably embolic from the heart. Thrombi were not found in the anterior descending coronary artery below the point of ligation. Two animals had left ventricular necrosis extending much farther laterally than was general; in one of these, anomalous duplicate anterior descending coronary arteries had been included in the snare. A small and strictly lateral infarction of the left ventricular wall was found in one animal, which showed ECG pattern (b) (Cf. supra).

Necrosis of the inner cortex of the adrenal was found in 2 animals; paracentral necrosis of the liver and splenic infarcts were found in 3 others. Various degrees of renal tubular necrosis were common. The anatomical changes ranged from necrosis of scattered groups of tubules to wedge-shaped areas of cortical necrosis. In some cases, these necroses were clearly older than the postinfarction time.

Series II

Two of the 17 animals undergoing surgery died postoperatively before ligation. In 2 of the 15 survivors, the ligation was unsuccessful because misplacement of the snare excluded the coronary artery or included part of the pulmonary valve. Nine of the monkeys died within 8 hours from myocardial infarction with a syndrome resembling cardiogenic shock; the 4 surviving animals were terminated on subsequent days. Because of the unequal and small size of the 2 groups, results are presented together for all monkeys.

Electrocardiogram. The ECG was similar to that previously described under pattern (a). Right bundle branch block was observed in 3 animals. Premature ventricular beats as well as ventricular fibrillation were common immediately after coronary occlusions. The episodes of ventricular fibrillation were successfully terminated with D.C. countershocks.

Hemodynamic and Blood Gas Studies. Immediately after ligation, aortic blood pressure and aortic flow decreased (Figure 5). Although some fluctuations occurred, the blood pressure and flow generally remained at these reduced levels.

Fifteen minutes after infarction, cardiac output decreased and the decline was still evident 45 minutes later (Table II). There was little

TABLE II.—*Cardiac Output and Blood Gas Analyses in Monkeys Injected with Microspheres (mean ± S.D.; N = 13)*

	Control Mean	15 min. After Infarction Mean	45 min. After Infarction Mean
Cardiac output (ml/min)	1106 ± 205	665 ± 200†	606 ± 50†
Cardiac output (ml/min/kg)	166 ± 34	99 ± 29†	89 ± 21†
Arterial pH	7.485 ± 0.031	7.494 ± 0.031	7.480 ± 0.052
Arterial CO_2 tension (mm. Hg)	37 ± 6	32 ± 6	32 ± 7
Arterial O_2 tension (mm. Hg)	103 ± 13	111 ± 11	108 ± 11

† Significantly different from control $p<0.001$
The Student "t" test for paired values was used.

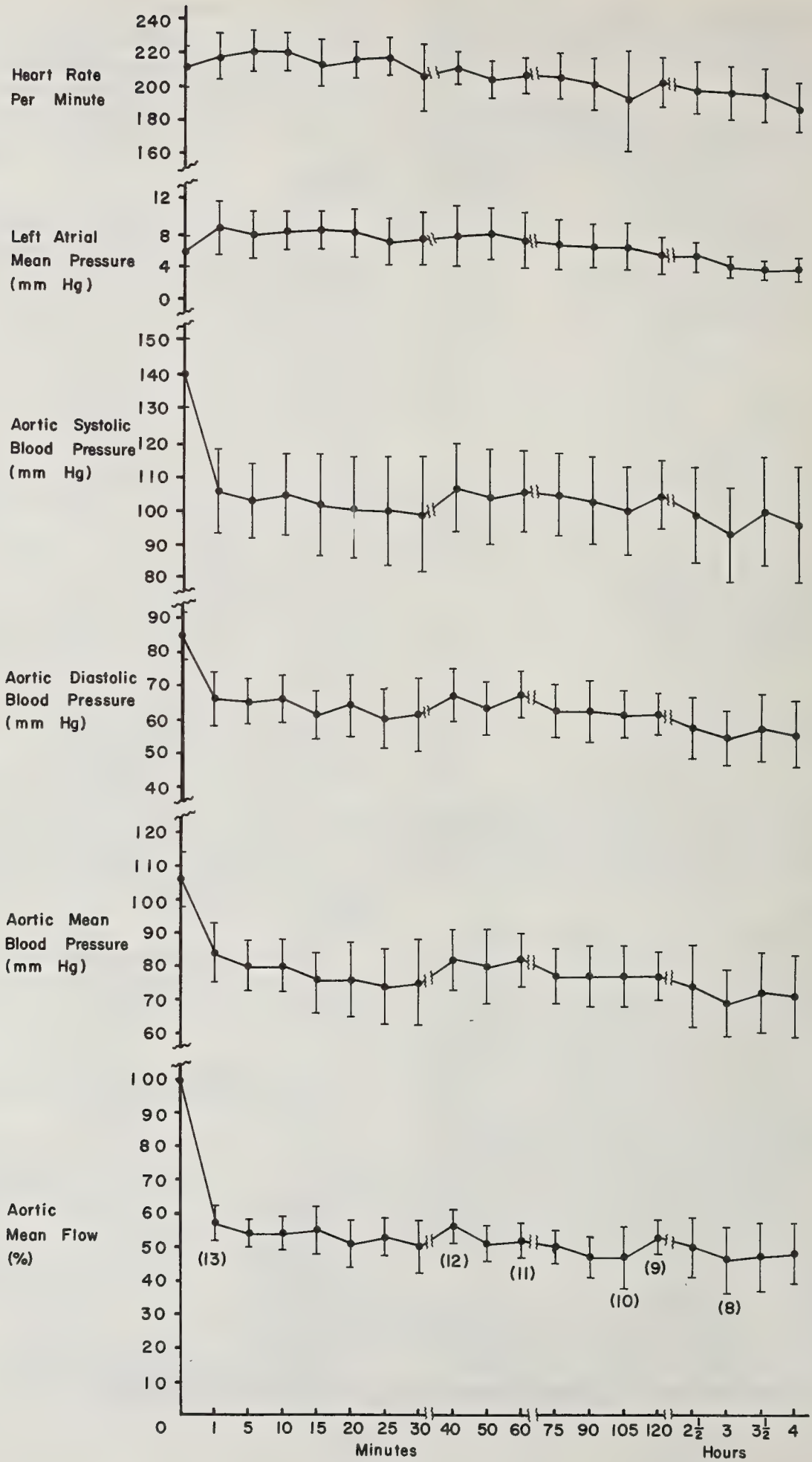


FIGURE 5.—Hemodynamic changes in monkeys after ligation of the anterior descending coronary artery (Series II). Symbols are same as in Figure 4.

change in blood gas and pH values before and after infarction. However, these determinations were not performed during the later stages of shock where differences might have been apparent.

Percent Distribution of Cardiac Output. Fifteen minutes after infarction, the percent of cardiac output perfusing the midbrain, brainstem, total brain, right atrium, and right ventricle was significantly increased; the fraction directed to the pancreas, spleen, mesentery, and thyroid was significantly reduced (Table III). The changes in percentage distribution to other organs were not statistically significant.

Forty-five minutes after infarction, there was a significant increase in the percentage distribution of cardiac output to the liver, right atrium, left atrium, and right ventricle and decreases to the pancreas, spleen, and mesentery.

Organs were divided into 2 groups according to the changes in the proportion of cardiac output delivered to them (Table IV). Group A included the brain, heart, liver, and adrenals where the percentage distribution of total car-

TABLE III.—*Blood Flow Distribution (mean ± S. D.; N = 13)*

Organ	Percent Cardiac Output Distributed to Each Organ			Organ Blood Flow (ml/min/100 g		
	Control Mean	15 min. After Infarction Mean	45 min. After Infarction Mean	Control Mean	15 min. After Infarction Mean	45 min. After Infarction Mean
Cerebral hemispheres	4.94 ± 2.30	6.31 ± 2.31	4.86 ± 2.17	69 ± 31	51 ± 17*	35 ± 12‡
Midbrain	0.16 ± 0.08	0.24 ± 0.08†	0.26 ± 0.13	74 ± 56	58 ± 20	59 ± 32
Brainstem	0.09 ± 0.06	0.15 ± 0.06†	0.16 ± 0.11	30 ± 19	28 ± 15	30 ± 22
Total Brain	5.99 ± 2.23	7.78 ± 2.38‡	6.41 ± 2.05	70 ± 25	53 ± 15‡	40 ± 10†
Pancreas	0.92 ± 0.34	0.42 ± 0.23‡	0.48 ± 0.32†	112 ± 45	30 ± 18‡	29 ± 16‡
GI Tract	6.96 ± 2.27	6.23 ± 2.95	6.90 ± 1.80	43 ± 15	25 ± 13‡	24 ± 11‡
Spleen	0.81 ± 0.67	0.27 ± 0.25†	0.42 ± 0.34‡	138 ± 83	30 ± 26‡	40 ± 28‡
Mesentery	1.05 ± 0.64	0.59 ± 0.52*	0.66 ± 0.42*	15 ± 15	5 ± 4‡	5 ± 6†
Liver (hepatic artery)	13.81 ± 3.67	16.00 ± 6.27	20.72 ± 6.50‡	90 ± 41	62 ± 33	72 ± 33
Total liver flow (hepatic artery and portal vein)	23.12 ± 4.15	23.55 ± 7.38	29.18 ± 6.92†	55 ± 24	35 ± 22†	37 ± 18†
Right Atrium	0.20 ± 0.11	0.32 ± 0.15‡	0.35 ± 0.25*	111 ± 62	112 ± 70	115 ± 91
Left Atrium	0.24 ± 0.22	0.33 ± 0.15	0.39 ± 0.27*	129 ± 90	115 ± 53	131 ± 104
Right Ventricle	0.77 ± 0.28	1.18 ± 0.50*	1.31 ± 0.84*	170 ± 68	151 ± 62	168 ± 145
Left Ventricle	5.06 ± 1.80	4.50 ± 0.77	4.74 ± 1.50	300 ± 118	157 ± 53†	151 ± 62†
Total Heart	6.32 ± 2.15	6.24 ± 0.88	6.83 ± 2.44	236 ± 82	139 ± 47†	139 ± 63†
Lungs	0.84 ± 0.51	0.58 ± 0.43	0.78 ± 0.82	18 ± 12	8 ± 7*	9 ± 10
Kidneys	13.47 ± 4.26	11.10 ± 5.19	11.09 ± 5.25	466 ± 171	231 ± 117‡	209 ± 113‡
Skin	5.62 ± 2.77	3.70 ± 1.20	4.04 ± 1.32	8 ± 4	4 ± 2†	3 ± 2‡
Carcass	43.46 ± 7.97	46.23 ± 9.48	40.82 ± 8.98	9 ± 3	6 ± 2†	5 ± 1†

* Significantly different from controls $p<0.05$
‡ Significantly different from controls $p<0.02$
† Significantly different from controls $p<0.01$
‡ Significantly different from controls $p<0.001$
The Student's "t" test for paired values was used.

TABLE IV.—*Comparison of Two Groups of Organs (mean ± S.E.)*

	Percent Distribution of Cardiac Output				
	Control	15 min. After Infarction	% Change	45 min. After Infarction	% Change
Group A*	26 ± 5.4	30 ± 6.3	+ 15	34 ± 8.4 +	+31
Group B**	74 ± 5.4	70 ± 6.3	−5	66 ± 8.4 Δ	−11
	Blood Flow (ml/min)				
	Control	15 min. After Infarction	% Change	45 min. After Infarction	% Change
Total Cardiac Output	1106 ± 205	665 ± 200 +	−40	606 ± 50‡	−45
Group A	295 ± 90	205 ± 77 Δ	−31	210 ± 81 ΔΔ	−29
Group B	811 ± 145	460 ± 139 +	−43	396 ± 106‡	−51

* Includes brain, heart, liver, and adrenals.
** Includes all remaining parts of the body.
Δ p (paired "t" test) <0.05
Δ $p<0.02$
† $p<0.01$
‡ $p<0.001$

diac output increased at the expense of Group B, which included the remaining parts of the body. Fifteen minutes after infarction, the cardiac output was redistributed so that Group A received a 15% increase whereas Group B received 5% less. The changes 45 minutes after infarction were more marked with Group A organs receiving a 31% relative increase in cardiac output at the expense of an 11% decrease to Group B.

Organ Blood Flow. Fifteen minutes after infarction, blood flow to the cerebral hemispheres and to the heart was significantly reduced; however, flow to the right ventricle and right and left atrium, which had not been significantly reduced 15 minutes after infarction, had returned to approximate control values 45 minutes after infarction. After both postinfarction microsphere injections, the blood flow was decreased to the remaining organs but not significantly to the liver and adrenal glands.

DISCUSSION

In our study, an extensive myocardial infarction was produced after ligation of the left anterior descending coronary artery similar to that observed in monkeys[14,15,17] and in other species.[22-24] The advantages of the closed-chest method in a nonanesthetized animal recommend it over the open-chested, anesthetized preparations in which marked changes in the cardiovascular system[25] are sometimes superimposed on the altered hemodynamics associated with positive pressure respiration. However, "chair" restraining of nonanesthetized animals may be responsible for additional artifacts not found, for example, in trained dogs in which myocardial infarction has been induced without anesthesia or restraining devices.[26-28]

Although ventricular fibrillation has been studied after acute coronary occlusion,[29-31] multiple episodes of ventricular fibrillation in the same animal have been rare. In our observations, continuous electrocardiographic recordings demonstrated the common occurrence of multiple episodes of ventricular fibrillation that were successfully terminated by the application of external D.C. electrical current.

In most studies, sinus tachycardia has been observed after myocardial infarction;[23,26,31] however, in our monkeys no difference in the heart rate was observed before and after coronary ligation. Since the control heart rate was over 200 beats/min and much higher than that reported in isolated chair-restrained monkeys,[32] our animals may have been under considerable sympathetic discharge and may not have reached basal values; conditioning to the restraining chairs and more rigid isolation should probably be considered in planning future experiments.

Several criteria have been proposed to define cardiogenic shock[33-36] including: (1) a fall in mean arterial pressure of at least 30% with no recovery for at least 30 minutes; (2) ischemic changes in the ECG with no arrhythmias to account for the fall in blood pressure; (3) a reduction of 50% of the cardiac output. These criteria were met by those animals of Series I that died within 6½ hours after myocardial infarction and, consequently, the diagnosis of cardiogenic shock can probably be sustained in the Group I ("Dead") group in spite of a lack of progressive rise in heart rate and end diastolic left ventricular pressure. The discriminant analysis based on data obtained in the first 30 minutes after infarction predicted the death or survival of the animals retrospectively. Thus, the present model may make possible studies on the therapy of cardiogenic shock that would be useful in managing this condition in man.

The validity of studies on regional blood flows by the use of nuclide-labeled microspheres is well established.[37,38] No significant differences in the percentage distribution of cardiac output nor in individual organ flows were observed between monkeys that presumably died in cardiogenic shock and those that survived. Although the small number of observations and the large differences between animals may account for this, another factor to consider in the interpretation of our findings is the probable high levels of sympathetic discharge evidenced by the tachycardia observed in the control periods. A pattern commonly observed, though, was a redistribution of the cardiac output favoring the brain, heart, liver, and adrenals so that in spite of the actual reduction in organ blood flow, these organs were less affected than the remaining parts of the body.

ACKNOWLEDGMENTS

We gratefully acknowledge the aid of Dr. C. W. de Lannoy, Jr. and Dr. Aleksander Knezevich in helping to devise some of the techniques of the earlier experiments. The authors are also indebted to Mr. Wm. Baughman, Mr. P. Madigan, and Mr. J. Lindsley for technical assistance; to Mr. D. Lindgren for writing the computer program, and Mr. J. Ito for illustrations.

REFERENCES

1. Malinow, M. R. Atherosclerosis in subhuman primates. Folia primat. 3:277–300, 1965.
2. McNulty, W. P., Jr., and Malinow, M. R. The cardiovascular system in: *Pathology of Primates* (ed.) R. N. Fiennes (in press).
3. Hamerton, A. E. Report on the deaths occurring in the society's gardens during the year 1930. Proc. zool. Soc., Lond. 527–555, 1931.
4. Hamerton, A. E. Report on the deaths occurring in the society's gardens during the years 1939–1940. Proc. zool. Soc., Lond. 111B: 151–185, 1941.
5. Hamerton, A. E. Report on the deaths occurring in the society's gardens during the year 1944. Proc. zool. Soc., Lond. 115:371–384, 1945–46.
6. Manning, G. W. Coronary disease in the ape. Am. Heart J. 23:719–724, 1942.
7. Burlingame, E. Annual report of the research and hospital department. Zoonos. Res. 18:6, 1945.
8. Ratcliffe, H. L. Report of Penrose Laboratory, Zoo. Soc. Philadelphia, p. 13, 1961.
9. Ratcliffe, H. L. Report of Penrose Laboratory, Zoo. Soc. Philadelphia, p. 12–13, 1961.
10. Taylor, C. B., Patton, D. E., and Cox, G. E. Atherosclerosis in rhesus monkeys. VI. Fatal myocardial infarction in a monkey fed fat and cholesterol. Arch. Path. 76:404–412, 1963.
11. Andrus, S. B., Portman, O. W., and Riopelle, A. J. Comparative studies of spontaneous and experimental atherosclerosis in primates. II. Lesions in chimpanzees including myocardial infarction and cerebral aneurysms. Progr. Biochem. Pharmacol., 4:393, Karger Basel/N.Y., 1968.
12. Hirsch, C., and Spalteholz, W. Coronararterien and Herzmuskel, Deutsche med. Wchnschr. 20:790, 1907 (from deWaart et al.).
13. Sutton, D. C., and Lueth, H. C. Experimental production of pain on excitation of the heart and great vessels. Arch. Int. Med. 45:827, 1930.
14. deWaart, A., Storm, C. J., and Koumans, A. K. J. Ligation of the coronary arteries in Javanese monkeys. I. Introduction, general experimental results, especially the changes in the ventricular electrocardiogram. Am. Heart J. 11:676, 1936.
15. Malinow, M. R., de Lannoy, C. W., Macpherson, C. H., and McNulty, W. P., Jr., Continuously monitored myocardial infarction in nonrestrained monkeys. Second International Congress of Primatology, Atlanta, Ga., p. 17, 1968.
16. Malinow, M. R., Kittinger, G. W., Maruffo, C. A., and de Lannoy, C. W., Jr. Dano miocárdico reversible en el *"Macaca mulatta"* producido por el isoproterenol. [Reversible myocardial damage in *Macaca mulatta* induced by isoproterenol.] Rev. Arg. Cardiol. 34:187, 1967.
17. Hill, J. D., Malinow, M. R., McNulty, W. P., Jr., and Ochsner, A. J., III. Experimental myocardial infarction in unanesthetized monkeys. Am. Heart J. 84:82–94, 1972.
18. Hill, J. D., Lees, M. H., Malinow, M. R., Ochsner, A. J., III, and Thomas, C. Regional blood flows in unanesthetized Rhesus monkeys during myocardial infarction. Cardiovascular Res. (in Press).
19. Anderson, T. W. *An introduction to multivariate statistical analysis.* John Wiley and Sons, Inc., N. Y., 1958.
20. Rudolph, A. M., and Heymann, M. A. The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow. Circulation Res. 21:163, 1967.
21. Malinow, M. R. An electrocardiographic study of *Macaca mulatta.* Folia primat. 4:51–65, 1965.
22. Hammer, J., and Pisa, Z. A method of isolated, gradual occlusion of a main branch of a coronary artery in closed-chest dogs. Am. Heart J. 64:67, 1962.
23. Hood, W. B., Jr., Covelli, V. H., and Norman, J. C. Acute coronary occlusion in pigs: effects of acetyl strophanthidin. Cardiovasc. Res. 3:441, 1969.
24. Lluch, S., Moguilevsky, H. C., Pietra, G., Schaffer, A. B., Hirsch, L. J., and Fishman, A. P. A reproducible model of cardiogenic shock in the dog. Circulation 39:205, 1969.
25. Forsyth, R. P., and Hoffbrand, B. I. Redistribution of cardiac output after sodium pentobarbital anethesia in the monkey. Am. J. Physiol. 218:214, 1970.
26. Rushmer, R. F., Watson, N., Harding, D., and Baker, D. Effects of acute coronary occlusion on performance of right and left ventricles in intact unanesthetized dogs. Am. Heart J. 66:522–531, 1963.
27. Noble, M. I. M., Trenchard, D., and Guz, A. Left ventricular ejection in conscious dogs: 1. Measurement and significance of the maximum acceleration of blood from the left ventricle. Circulation Res. 19:139–147, 1966.
28. Chimoskey, J. E., Szentivanyi, M., Zakheim, R., and Barger, A. C. Temporary coronary occlusion in conscious dogs: collateral flow and electrocardiogram. Am. J. Physiol. 212:1025–1032, 1967.

29. Weisse, A. B., Moschos, C. B., Passannante, A. J., Khan, M. I., and Regan, T. J. Relative effectiveness of three antiarrhythmic agents in the treatment of ventricular arrhythmias in experimental acute myocardial ischemia. Am. Heart J. 81:503, 1971.
30. Harris, A. S. Delayed development of ventricular ectopic rhythms following experimental coronary occlusion 1:1318, 1950.
31. Clark, B. B., and Cummings, J. R. Arrhythmias following coronary occlusion and their response to drugs. Ann. N. Y. Acad. Sci. 64:543, 1956.
32. Stahl, W. R., and Malinow, M. R. A survey of physiological measurements in *Macaca mulatta*. Folia primat. 7:12–33, 1967.
33. Agress, C. M., Rosenberg, M. J., Jacobs, H. I., Binder, M. J., Schneiderman, A., and Clark, W. G. Protracted shock in the closed-chest dog following coronary embolization with graded microspheres. Am. J. Physiol. 170:536, 1952.
34. Agress, C. M. Pathogenesis and management of coronary shock. Geriatrics 21:194, 1966.
35. Perlroth, M. G., and Harrison, D. C. Cardiogenic shock: A review. Clin. Pharmacol. Therap. 10:449, 1969.
36. David, M. S., Charrette, E. J. P., and Lynn, R. B. Experimental coronary artery thrombosis for production of cardiogenic shock. Canada J. Surg. 13:189, 1970.
37. Neutze, J. M., Wyler, F., and Rudolph, A. M. Use of radioactive microspheres to assess distribution of cardiac output in rabbits. Am. J. Physiol. 215:486, 1968.
38. Forsyth, R. P., Nies, A. S., Wyler, F., Neutze, J., and Melmon, K. L. Normal distribution of cardiac output in the unanesthetized, restrained rhesus monkey. J. Appl. Physiol. 25:736, 1968.
39. Hoffbrand, B. I., and Forsyth, R. P. Validity studies of the radioactive microsphere method for the study of distribution of cardiac output, organ blood flow, and resistance in the conscious rhesus monkey. Cardiovasc. Res. 3:426, 1969.

DISCUSSION

Chairman La Farge: Thank you Dr. Malinow. The floor is open to discussion. I'd like to question Dr. Malinow a little more about the clinical applicability of the model in question. This is a very neat model. It uses aortic, systolic, diastolic and mean pressures, but unfortunately, from a clinical standpoint, calls upon aortic flow which is very difficult, if not impossible to measure in the post-operative surgical patient, unless one uses, for example, cardiac green dye curves. Can you do without the flow term? Have you tried to apply this clinically?

Dr. Malinow: Yes Dr. LaFarge. I tried here to show our best prediction which was 100% for the cardiogenic shock but we also did the same study without using flow and we obtained almost the same result. We were able to predict almost 80% of the outcome in cases of cardiogenic shock just by using pressure.

Sidney Cassin, University of Florida: I think you indicated that you used 50,000 spheres for injection and somewhere 100 to 150,000. Is this correct? Did you notice any effect of the spheres on the myocardium per se? Was there any plugging that was obvious in terms of ECG or pathology post-injection—since you initially produced an ischemia and then injected the spheres? Is this correct?

Dr. Malinow: Microspheres did not change the hemodynamics of the preparation. As you know the method for the detection of the spheres is by calcination of the whole tissues; obviously, we could not do a histological study. So we have not done histological studies in the animals that were injected with the microspheres, and we were not able to detect any obvious hemodynamic differences as compared to those which have not been injected with microspheres.

Chairman: I'd like to add a comment: most people who have used single or multiple isotopes for coronary flow distribution studies, use them in such small quantities that in our hands and in the hands of Julian Hoffman on the West Coast, we have certainly not seen any changes in myocardial performance. The question is: is this a double insult? In order of magnitude, the insult of the snare is at least greater, I suspect, than the insult of the beads.

CANINE CORONARY INSUFFICIENCY MODELS FOR EVALUATING SYSTEMS FOR CIRCULATORY SUPPORT*

J. Rosensweig and S. Chatterjee*

Canine experimental models of coronary insufficiency will be described. Acute and chronic models were utilized in studying effects of counterpulsation on coronary collateral circulation, circulatory dynamics, myocardial function and survival.

Chronic insufficiency models were created by placement of ameroid constrictors about the origin of left coronary arteries. The predominant left coronary circulation in dogs makes this preparation a rather severe test of potential collaterals. Within 14 days, flow declined to 49% with little reduction thereafter despite continuing proximal stenosis. A reproducible state of stable coronary insufficiency was achieved which facilitated assessment of counterpulsation-induced collateral circulation. Within one hour, retrograde flow could be demonstrated which correlated with angiographic findings, restoration of myocardial oxidative metabolism, reduced incidence of fatal arrhythmia and myocardial infarction and prolonged survival.

Acute insufficiency models, produced by serial ligation of branches of the anterior descending and circumflex arteries, were used to induce cardiogenic shock. Shock was characterized by sustained elevation of left atrial pressure above 12 mm. Hg., reduction of cardiac output by at least 40% and a 30% or more fall in aortic systolic pressure. Untreated, the animals died from progressive left ventricular failure and fibrillation within 135 minutes. This interval allowed sufficient time for institution of circulatory support by counterpulsation and permitted study of three different modalities—arterio-arterial counterpulsation, intra-aortic balloon pumping and venoarterial bypass with oxygenation and diastolic augmentation. The acute model served effectively to indicate the advantages and limitations of each form of therapy.

INTRODUCTION

Experimental canine models were used to investigate effects of counterpulsation in chronic coronary insufficiency and acute cardiogenic shock. These models were readily reproducible and closely simulated corresponding clinical situations.

*Lady Davis Institute For Medical Research, Jewish General Hospital, Montreal, Canada

Present address, Departments of Surgery, University of Connecticut School of Medicine and Mount Sinai Hospital, Hartford, Connecticut 06112.

STUDY I

This study was designed to evaluate counterpulsation-induced collateral circulation, in relation to survival, coronary flow and myocardial metabolism. Three different experiments were carried out utilizing the chronic coronary insufficiency preparation initially described by Litvak and Vineberg.[1] In each experiment, counterpulsation was carried out for one hour on the 14th day, a time corresponding to the development of at least 50% luminal constriction—i.e. critical coronary artery stenosis. Arterio-arterial counterpulsation using the Simas pump was instituted by cannulation of the femoral artery (Figure 1A). The aspiration phase of the pressure pulse generator was begun in presystole and continued throughout systole. Blood was returned to the arterial circulation during diastole. Pump stroke volume of 10–15 cc. achieved the desired alteration in aortic pressure configuration.

Experiment I—Survival Study

Twenty-four adult mongrel dogs were operated upon, half serving as control. By left thoracotomy, chronic progressive coronary insufficiency was induced by application of a paraffin coated formalized casein (ameroid) constrictor 2.77 mm. I.D. about the origin of the anterior descending artery (just distal to the takeoff of the septal artery) and about the origin of the circumflex branch of the left coronary artery (Figure 2).

Following placement of constrictors, the animals were allowed to recover. Dogs in the treated group were subsequently counterpulsated on the 14th day. All animals were followed until death, but long-term survivors were sacrificed after 90 days. At autopsy, the degree of

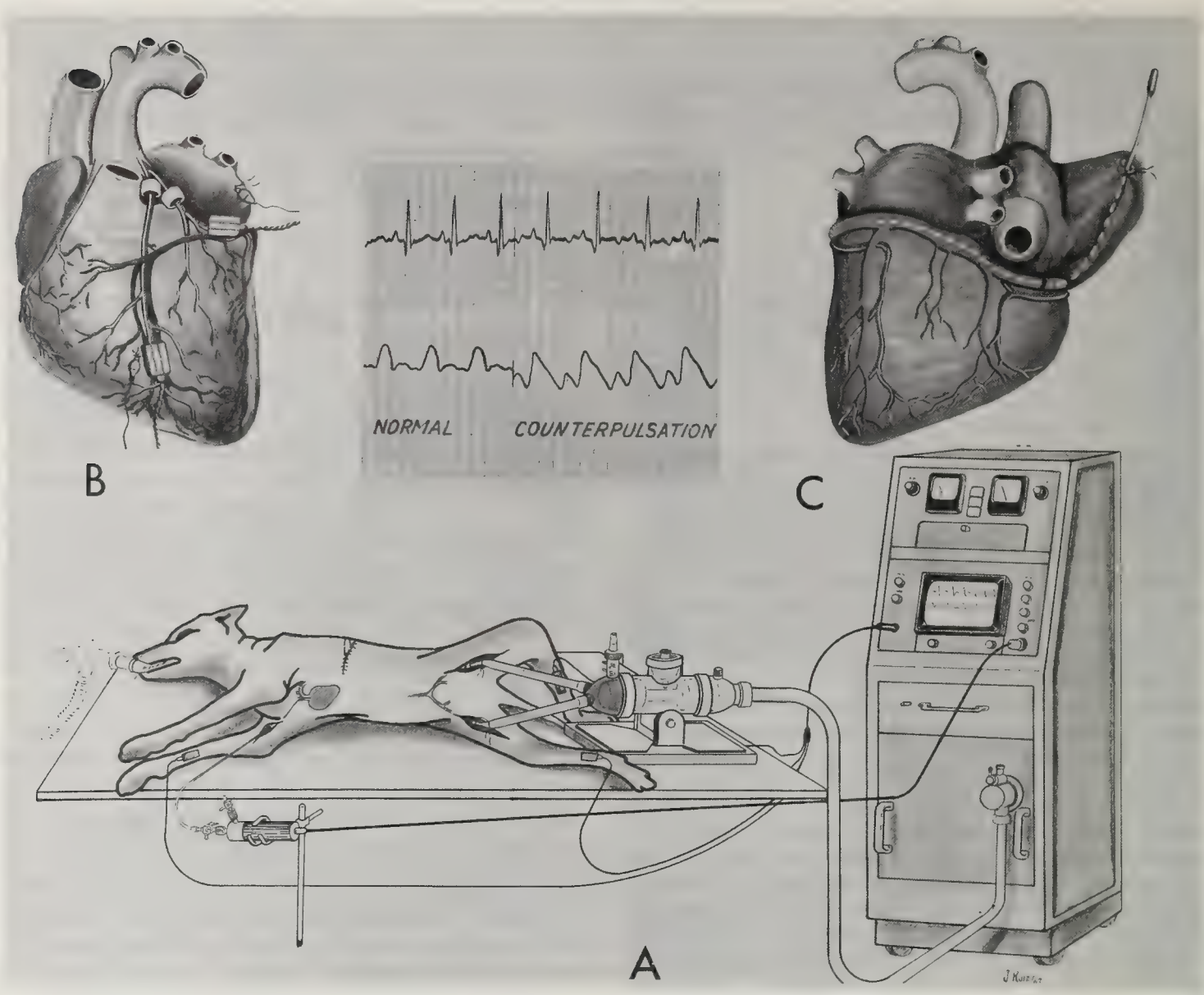


FIGURE 1.—a. Operative schema for arterio-arterial counterpulsation. Note reduction of aortic systolic pressure and augmentation of diastolic pressure during pumping. b. Insert indicating implantation sites of constrictors and flow probes about the anterior descending and circumflex arteries. c. Insert demonstrating technique of implanting the coronary sinus catheter.

coronary artery constriction was determined, coronary arteriograms were made according to the technique of Robbins[2] and myocardial sections taken for histologic examination.

Experiment II—Hemodynamic Study

Twenty-three mongrel dogs were operated upon. All had coronary artery constrictors implanted as in Experiment I. In the control group, an electromagnetic flow probe was placed about both coronary arteries 3–4 centimeters distal to the constrictors in 4 dogs and a single probe placed about the circumflex artery in 7 animals (Figure 1B). In the treated group, flow probes were placed about both arteries in 6 dogs and a single probe about the left circumflex artery in 6 animals. The coronary blood flow was carefully monitored. Treated dogs were counterpulsated on the 14th day and followed until death or sacrifice after 30 days. Autopsy examination was then carried out.



FIGURE 2.—Angiograms of counterpulsated dogs showing (a) no intercoronary collateral circulation: however, marked homocoronary circulation from prominent septal and anterior left ventricular diagonal arteries (b).

Experiment III—Metabolic Study

Nine adult mongrel dogs were operated upon. Coronary artery constrictors were placed as in Experiment I. Blood samples were drawn from the aorta and coronary sinus for lactate and pyruvate concentration. On the 14th day, by right thoracotomy, a cannula penetrating the right atrial wall was inserted into the coronary sinus and its distal end brought out through the chest wall (Figure 1C). Following closure of the incision, the animal was counterpulsated. Aortic and coronary sinus blood samples were drawn immediately prior to and following counterpulsation for lactate and pyruvate concentration. The coronary sinus lactate concentration was then repeated daily for three days.

RESULTS

Experiment I

Seven of the nine control animals died within thirty days. The other two died on the thirty-second and forty-fifth day, respectively. Mean survival in control animals was 23.5 days. However, all treated dogs survived the one month period. Two animals died on the thirty-first day and thirty-seventh day and eight animals lived beyond ninety days and were later sacrificed. Mean survival to time of death or sacrifice in the treated group was 91.3 days. The difference in mean survival between the untreated and counterpulsated animals was strikingly significant ($t = 6.5$, $P < 0.001$).

Mean coronary artery constriction at time of death in control animals was 66 percent and 65 percent in the anterior descending and circumflex artery, respectively; whereas treated dogs revealed 93 percent and 93 percent, respectively. Postmortem angiograms were assessed in terms of collateral circulation to the distal branches of proximally occluded anterior descending and circumflex arteries. Three primary sources for collateral circulation were apparent—the right coronary artery, the septal artery, and diagonal anterior left ventricular branches arising directly from the left main coronary artery proximal to the constrictors.

In the control group, eight of the nine dogs revealed no intercoronary collateral circulation, whereas only two of the ten counterpulsated animals showed no right to left intercoronary anastomotic vessels. In six treated dogs, there was excellent intercoronary circulation. Homocoronary collateral circulation was also more obvious in the counterpulsated group. The septal artery was prominent in every treated dog with numerous arborizing vessels communicating with branches of the anterior descending and circumflex arteries. In addition, diagonal anterior left ventricular arteries arising proximal to the constrictors (not seen in control animals) also communicated with branches of the anterior descending and circumflex arteries (Figures 2 and 3).

Histologic examination revealed increased prominence and number of subepicardial as well as intramyocardial arteries and arterioles in treated dogs. Myocardial infarction was not detected in the hearts of counterpulsated animals, but was observed in five of the nine control animals.

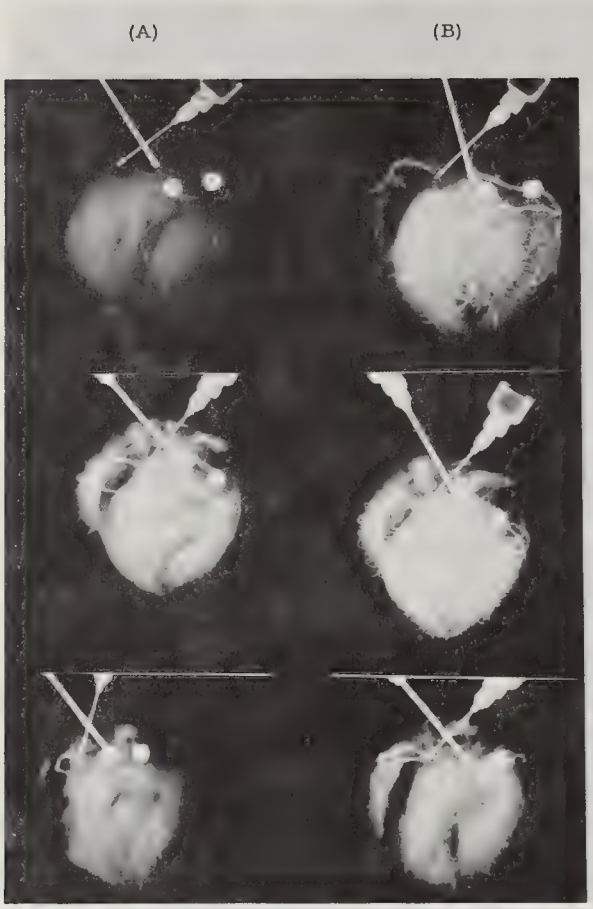


FIGURE 3.—Angiograms of counterpulsated dogs revealing (a) retrograde filling on the left coronary artery branches by injection of the right coronary artery. Injection of the left coronary artery (b) reveals marked homocoronary collateral circulation from prominent septal and anterior diagonal left ventricular branches arising proximal to the constrictors.

Experiment II

Following placement of constrictors, there was progressive fall in coronary flow, most marked in the first ten days. In control animals, the flow fell to an average of 50 percent of initial reading by the fourteenth day, with little drop thereafter, despite increasing proximal stenosis (Figure 7). At the final determination prior to death, the flow averaged 41 percent. Coronary flow increased after the fourteenth day in only one animal and the rise was minimal.

In treated animals, a mean drop in coronary flow to 50 percent on the fourteenth day was also observed, but counterpulsation produced dramatic restoration to normal or slightly above initial levels (Figure 8). The rise did not develop immediately, but was apparent within thirty minutes. Coincident with the rise in coronary flow, there was reversal of polarity indicating reversal of direction of blood flow (Figure 4). After cessation of pumping, the increased flow with reversed polarity persisted. The flow subsequently diminished gradually over several days, eventually stabilizing at an average of 89 percent of initial reading. Histograms comparing the findings in control and counterpulsated dogs are presented in Figure 5.

All untreated control animals died a sudden (presumably arrhythmic) death between thirty and thirty-six days after placement of constrictors. None of the counterpulsated dogs died, but were sacrificed between the thirtieth and thirty-sixth day. Similar mean coronary artery constriction was found at autopsy in

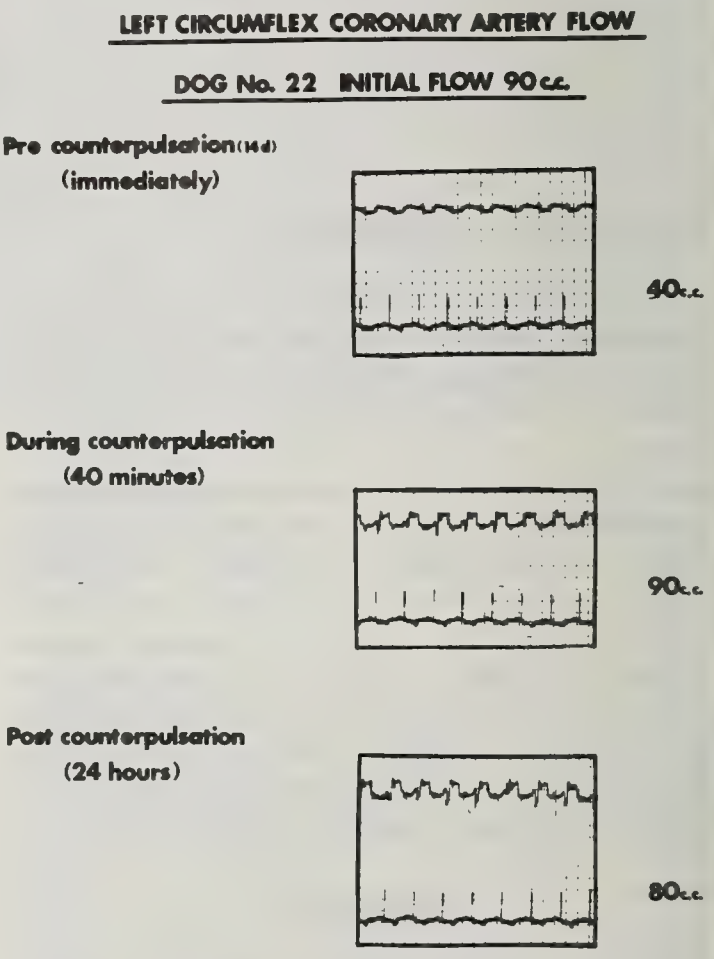


FIGURE 4.—Flow tracing demonstrating increased flow after forty minutes of counterpulsation. Amplitude of flow during diastole is increased and the polarity is reversed.

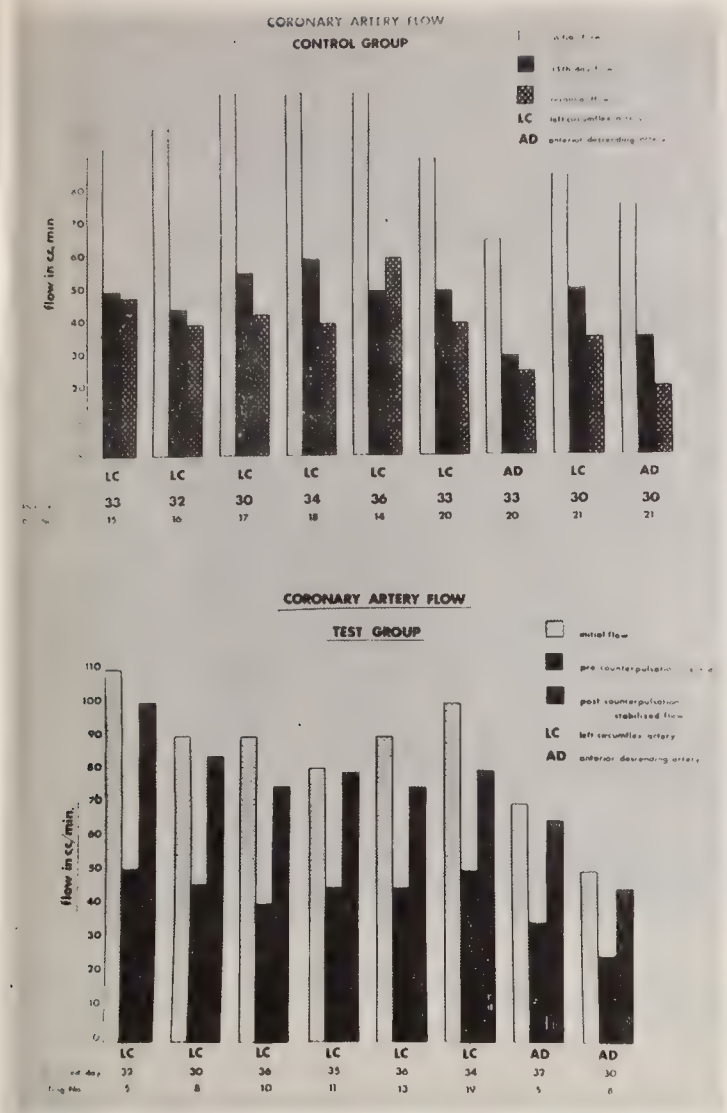


FIGURE 5.—Composite histograms of coronary flow in control dogs and animals treated by counterpulsation.

both groups, namely 74 percent and 73 percent, respectively in the anterior descending and circumflex artery in untreated controls and 76 percent and 76 percent, respectively, in the counterpulsated dogs.

Angiograms revealed retrograde filling of the occluded arteries from the same three sources observed in Experiment I. Retrograde filling of the anterior descending and circumflex artery systems was achieved through anastomotic branches of the right coronary artery and the septal artery. In counterpulsated dogs, both vessels were larger and showed more extensive arborization. Other sources of collateral flow were diagonal anterior left ventricular branches (arising proximal to the constrictors) that were not initially apparent when constrictors were placed. Four of the six treated dogs had good to excellent intercoronary collateral circulation but only one dog in the control group revealed intercoronary collateral flow (Figure 11). Similarly, homocoronary collateral circulation was profuse in all counterpulsated dogs, but in only two untreated animals (Figure 12).

Mycardial infarction was detected histologically in four of the seven untreated animals but was not observed in counterpulsated dogs. These observations correlated with increased number and prominence of subepicardial arteries, as well as of branching intramyocardial arteries and arterioles coursing through the septum and left ventricular myocardium in counterpulsated dogs (Figures 6 and 7).

Experiment III

Lactate concentration in the arterial and coronary sinus blood increased progressively following application of constrictors (Figure 8). Initially the lactate concentration was greater in the arterial blood (mean arterial concentration was 2.6 mM./L., coronary sinus 2.3 mM./L.). Comparison of initial and pre-counterpulsation values indicates that the increased levels of lactate concentration were significant (arterial $t = 2.88$, $P < 0.05$; coronary sinus $t = 4.6$, $P < 0.01$).

Following counterpulsation mean lactate concentration was reduced in both the samples (arterial 2.6 mM./L., coronary sinus 2.3 mM./L.), but the coronary sinus concentration was less than the arterial concentration. Coronary sinus lactate concentration three days post-counterpulsation remained low (2.3 mM./L.), not significantly different from the initial level.

Excess lactate was calculated according to Huckabee's formula.[3] Mean excess lactate initially was negative (—0.3 mM./L.), but on the fourteenth day prior to counterpulsation, it became positive (0.2 mM./L.), denoting anaerobic myocardial metabolism (Figure 16). Following counterpulsating, however, mean excess lactate became negative again (—0.1 mM./L.) indicating that the myocardium was no longer produc-

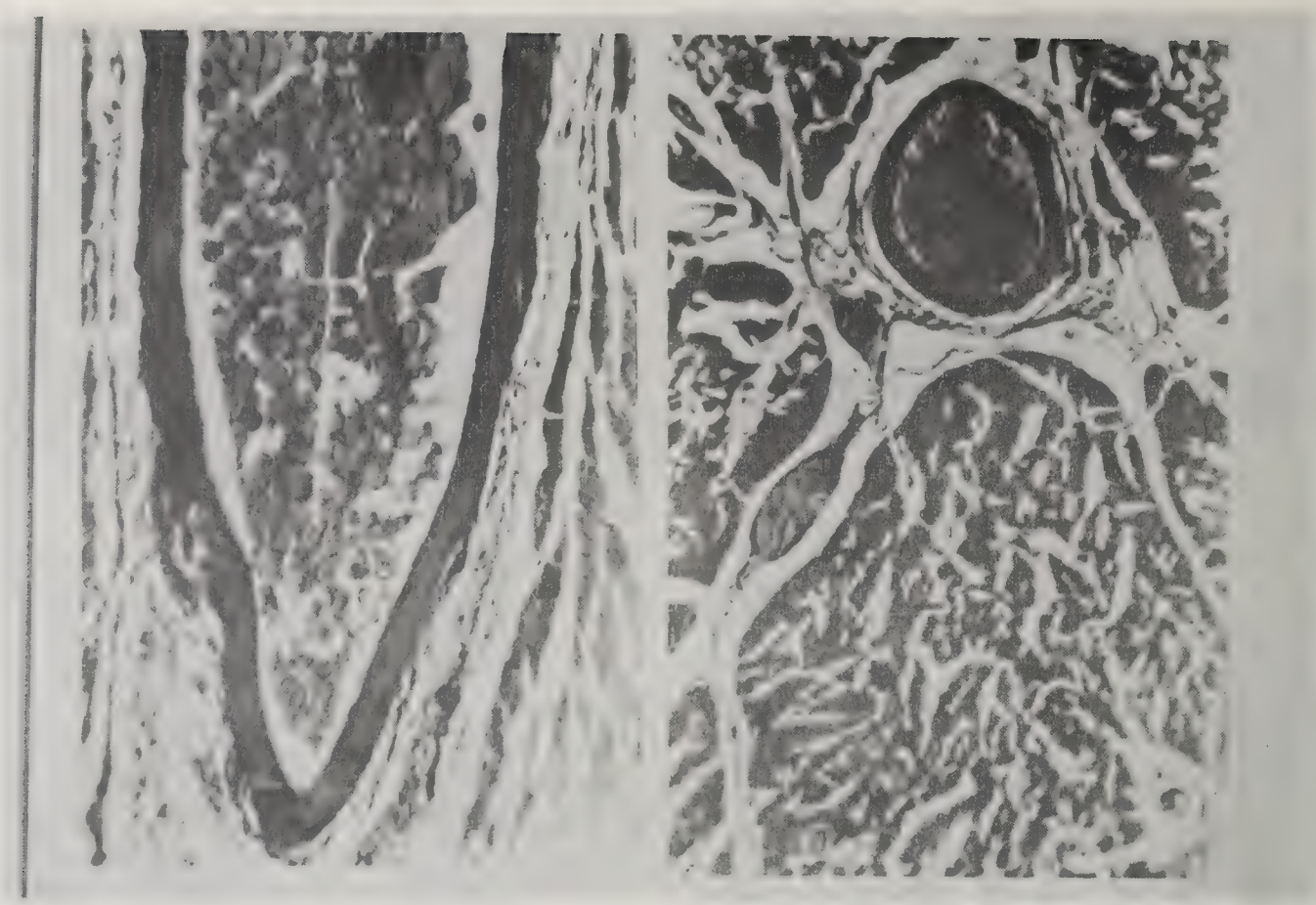


FIGURE 6.—Photomicrograph showing the calibre of the intramyocardial septal artery in dog No. 11 (a) and branching arterioles (b) in counterpulsated dog No. 8.

ing lactate but was extracting it from arterial blood.

Comments—Study I

The chronic coronary insufficiency preparation of Litvak and Vineberg[1] served as an excellent reproducible ischemic preparation for evaluating the effects of counterpulsation on coronary collateral circulation. As in acute ligation experiments, consistent effects on coronary blood flow depend upon proper placement of constrictors.[4,5] The constrictor must be placed about the anterior descending artery immediately distal to the takeoff of the septal artery and at the origin of the circumflex artery. Coating the formalized casein constrictor with paraffin slows the rate of fluid absorption so that when 2.77 mm. I.D. constrictors are used in 20 kilogram dogs, total occlusion of the coronary arteries does not occur before one month.[6] After ten to fourteen days, a relatively stable period of coronary insufficiency is produced despite progressive proximal coronary artery constriction. This basic ischemic heart model can then be readily adapted according to experimental design to hemodynamic or metabolic studies.

In Experiment I, 80% of control animals succumbed within one month, whereas 80% of the treated dogs survived and 60% lived long-term despite almost complete proximal occlusion of the anterior descending and circumflex arteries. The sudden death of control animals suggests a terminal fatal arrhythmic episode, probably ventricular fibrillation. The explanation for prolonged survival of treated animals was apparent in the postmortem angiograms. One must assume, therefore, that potential collateral channels exist within the heart. Burton has shown that vascular resistance in nonfunctional closed vessels is infinitesimally great and requires a critical opening pressure to overcome the surface and wall tension.[7] Increased

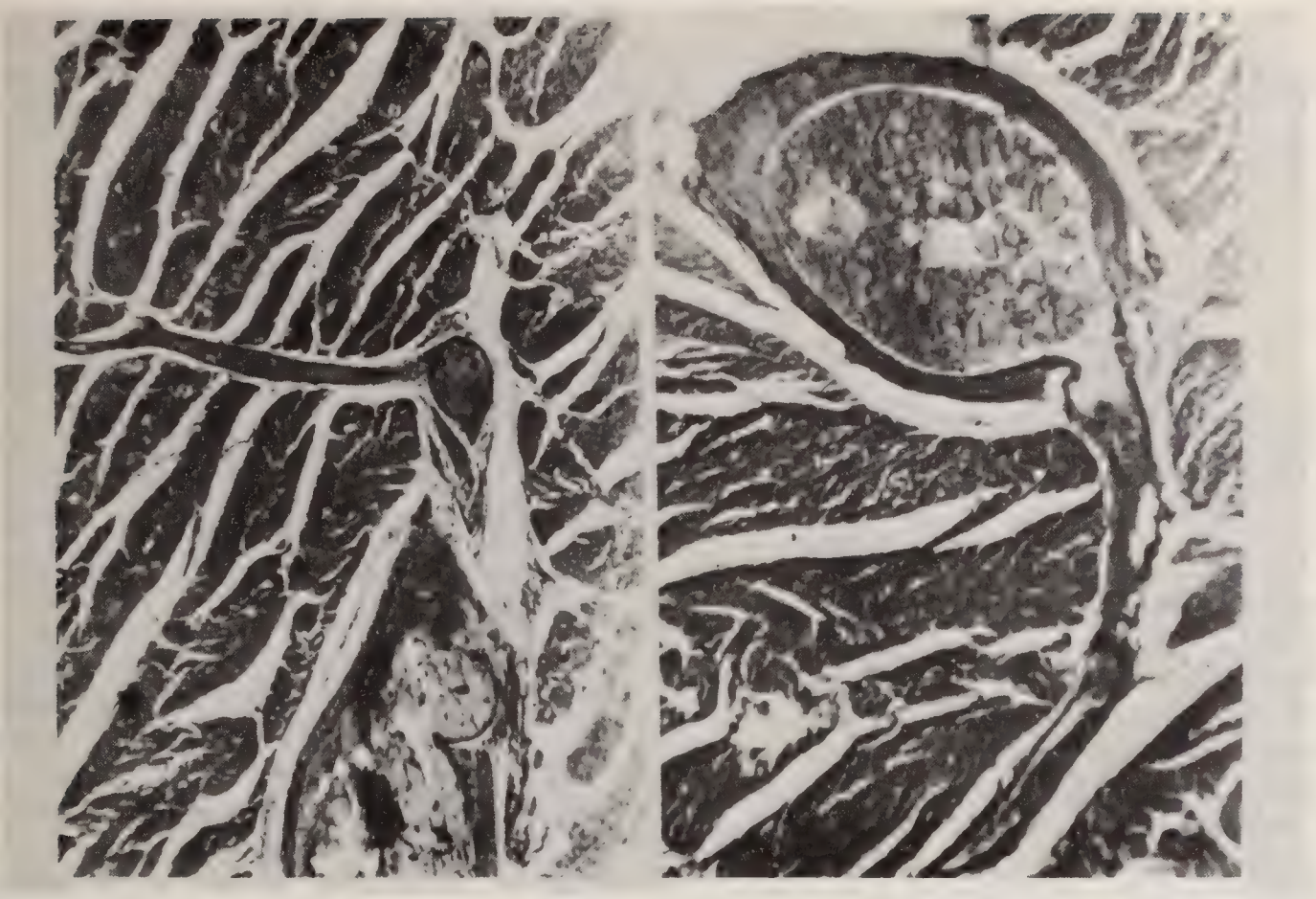


FIGURE 7.—Photomicrograph (low power) of intramyocardial arteries and arterioles in the left ventricular myocardium in dog No. 5 (a) and in dog No. 8 (b).

intraluminal pressure alters the transmural pressure and increases the vessel diameter. There is a dynamic relationship between flow, pressure, volume and caliber in distensible blood vessels. Since resistance is related to the inverse of the radius to the fourth power (Poiseuille's equation), resistance to flow can be rapidly diminished by increments above critical opening pressure. Beyond a certain point, the resistance in small vessels becomes constant and the flow is then solely related to the driving pressure.

In the ischemic heart, it is likely that counterpulsation particularly affects the caliber of small arteries and arterioles which communicate with distal branches of proximally occluded coronary arteries. Because of their lack of external support, epicardial vessels are especially vulnerable. The diameter of small vessels is increased and potential non-functioning channels are opened. Once collateral flow is established, it persists, even after cessation of pumping. Flow is then maintained by the pressure gradient across the ischemic bed and the resultant diminished vascular resistance is enhanced by the local metabolic milieu favoring vasodilation. The angiogram and histologic studies of long-term survivors suggest that with passage of time, the pattern of collateral circulation becomes prominently established as the new channels accommodate to the increased flow. A two phased process of transformation of a small myocardial arteriole into a functionally efficient intercoronary anastomotic artery in the dog has been previously described by Schaper and Vandesteene.[8]

The collateral circulation which developed naturally following ameroid constriction in untreated control animals was insufficient for adequate protection of the myocardium. The stabilized, although diminished, distal flow beyond the fourteenth day was interpreted as reflecting maximal natural development of collateral circulation in the ischemic heart. However, in

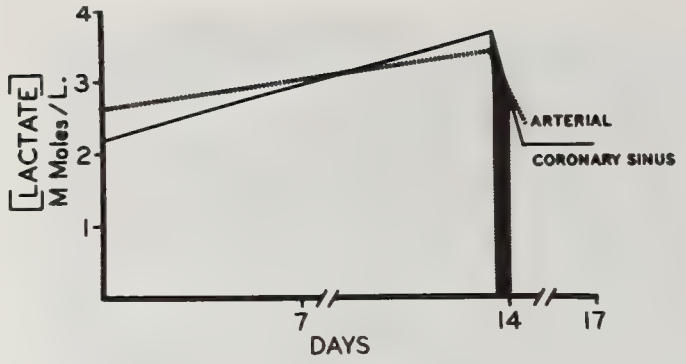


FIGURE 8.—Effect of counterpulsation on arterial and coronary sinus lactate concentration in the ischemic heart. Mean findings of five experiments.

treated dogs, within thirty minutes of counterpulsation there was an increase in coronary flow to near normal levels. Reversed polarity observed coincidence with a sharp rise in coronary flow supports the concept of mechanically induced retrograde circulation. There appears to be a critical opening time of thirty minutes for potential collateral vessels. Beyond this time, counterpulsation failed to produce any additional benefit. The persistence of elevated coronary flow with reversed polarity long after cessation of pumping correlated with postmortem angiographic patterns of retrograde circulation and with vinylite corrosion casts of coronary collateral circulation observed in an earlier study.[9]

With the onset of coronary insufficiency, lactate concentration rose in both the arterial and coronary sinus blood and the myocardium, instead of extracting lactate, began producing it (Experiment III). This was reflected in elevated concentrations of lactate in coronary sinus blood and the development of a positive excess lactate. However, following counterpulsation, there was a decline in lactate concentration in both the coronary sinus and arterial blood, but the coronary sinus lactate concentration was lower in the arterial sample, suggesting that normal oxidative metabolism was restored and providing additional evidence of increased myocardial perfusion.

Prolonged survival of treated animals can therefore be correlated with restoration of near normal perfusion to ischemic myocardium. It is undoubtedly related to prevention or restriction of the extent of myocardial infarction, the reduction of myocardial irritability and the return of normal myocardial oxidative metabolism and ventricular function.

STUDY II

This study was designed to evaluate the effect of three different methods of counterpulsation on circulatory dynamics and metabolism in acute cardiogenic shock. Adult mongrel dogs were used and a technique of serial coronary artery branch ligation was selected as the method for producing acute cardiogenic shock. The animals were divided into a control group and three different treatment groups. The dogs were all subjected to a left thoracotomy and placement of appropriate flow probes and cannulas for monitoring hemodynamic parameters and blood sampling (Figure 9A). The electrocardiogram, aortic pressure, left atrial pressure, cardiac output and coronary blood flow were monitored throughout each experiment. Serial determinations of arterial oxygen and lactate concentration were also obtained. In Groups 1 and 2, the coronary sinus lactate concentration and in Groups 3 and 4, the plasma hemoglobin concentration and hourly urine output were determined.

In the control group, once cardiogenic shock was induced, no treatment was administered and the animals were followed closely until death. However, in the three treatment groups, counterpulsation was instituted when cardiogenic shock became manifest. At the termination of each experiment, autopsy examination was carried out after death or sacrifice.

Cardiogenic Shock Model

Progressive left ventricular failure was produced by serial ligation of branches of the anterior descending and left circumflex coronary arteries. The branches were ligated at their origin as illustrated in Figure 9B. Usually, cardiogenic shock did not develop unless the two major diagonal branches of the anterior descending, the marginal artery and one or two proximal smaller branches of the left circum-

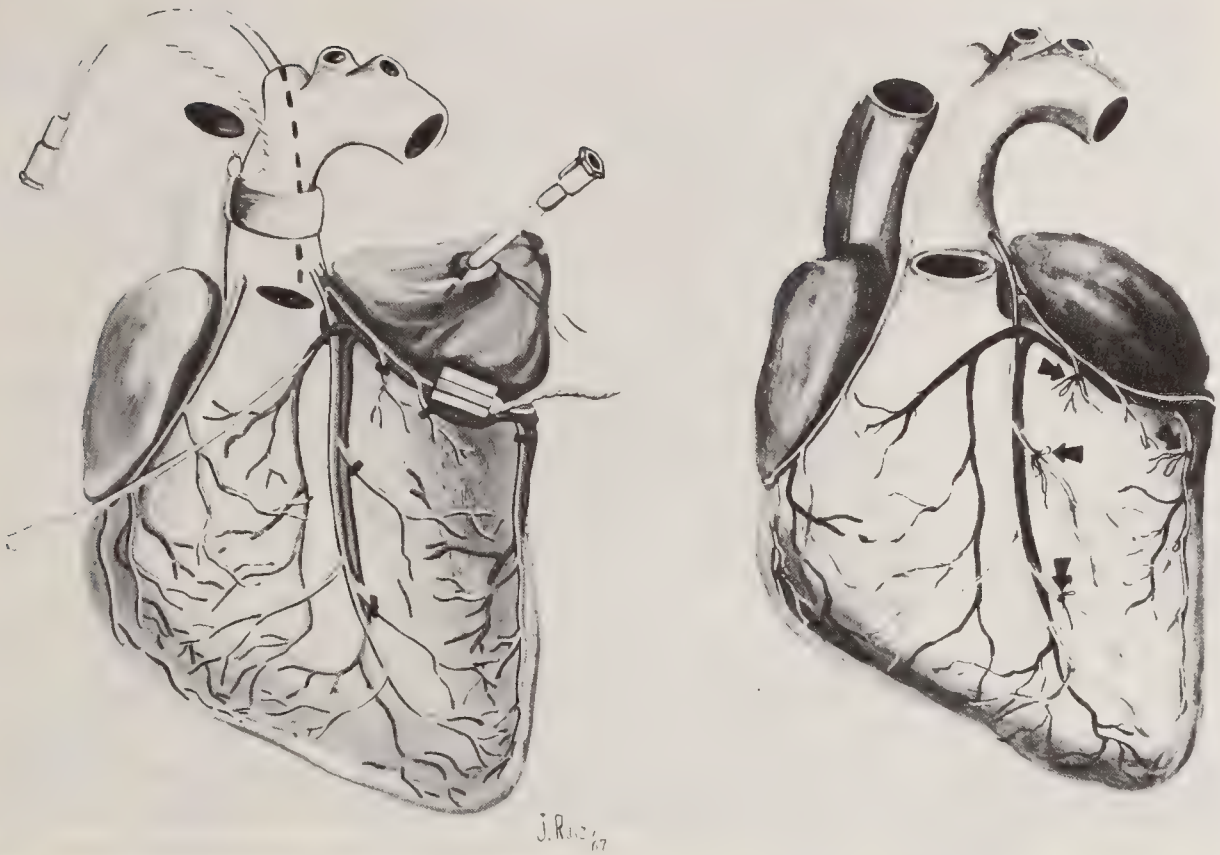


FIGURE 9.—a. Experimental preparation in Study II. b. Arrows indicate the ligated branches of the left coronary artery.

flex artery were ligated. The marginal branch of the left circumflex artery was ligated initially, then the major branches of the anterior descending artery. At least 15–30 minutes were allowed between ligations for stabilization of transient acute effects on coronary flow and hemodynamics.

Criteria for cardiogenic shock were:

1. Sustained elevation of mean left atrial pressure above 12 mm. Hg.
2. Reduction of cardiac output by 40 percent or more, and
3. Diminution in aortic systolic pressure by 30 percent or more.

The latter two criteria were invariably present when mean left atrial pressure remained above 12 mm. Hg. Time interval from ligation of the first coronary artery branch to the development of cardiogenic shock average 120 minutes (70–160 minutes in Group I, 138 minutes (116–170 minutes) in Group II, 137 minutes (110–160 minutes) in Group III, and 140 minutes (110–175 minutes) in Group IV. The time differences between the groups were not significant ($t = 1.464$, $p = 0.25$).

TREATMENT PROCEDURES

Group I

Ten animals served as controls. Once cardiogenic shock was manifest, the dogs were monitored until death.

Group II

Ten animals were subjected to arterio-arterial counterpulsation for one hour using a SIMAS pump. Counterpulsation was effected through No. 14 Fr. cannulas inserted into both femoral arteries as in Study I (Figure 1A). Because of

concern about excessive blood trauma and the possibility of reported deleterious effects upon peripheral circulation during prolonged counterpulsation,[10,11,12] counterpulsation was limited to one hour (a period found to be safe and well tolerated by dogs in Study I). The animals were carefully followed until death or sacrifice after twenty-four hours.

Group III

Six dogs were treated by intra-aortic balloon counterpulsation for four hours (Figure 10A). When pumping was discontinued, the animals were observed for one hour, then sacrificed.

A monosegmented balloon with a volume of 15 cc. was used. The balloon catheter was inserted through a femoral arteriotomy and positioned in the descending thoracic aorta under radiologic control. Preliminary experiments in normal adult dogs (20–30 Kgm) demonstrated that with this caliber balloon (fully inflated and just subocclusive), intra-aortic balloon counterpulsation was well tolerated for at least four hours without evidence of thrombosis, embolization or local aortic injury.[13] Balloon counterpulsation reduced systolic pressure an average of 15 percent, augmented diastolic pressure 44 percent (Figure 10B) and diminished left ventricular work 17 percent while coronary, carotid and renal flow was maintained. The hourly increment of plasma hemoglobin was 6 mgm. percent.

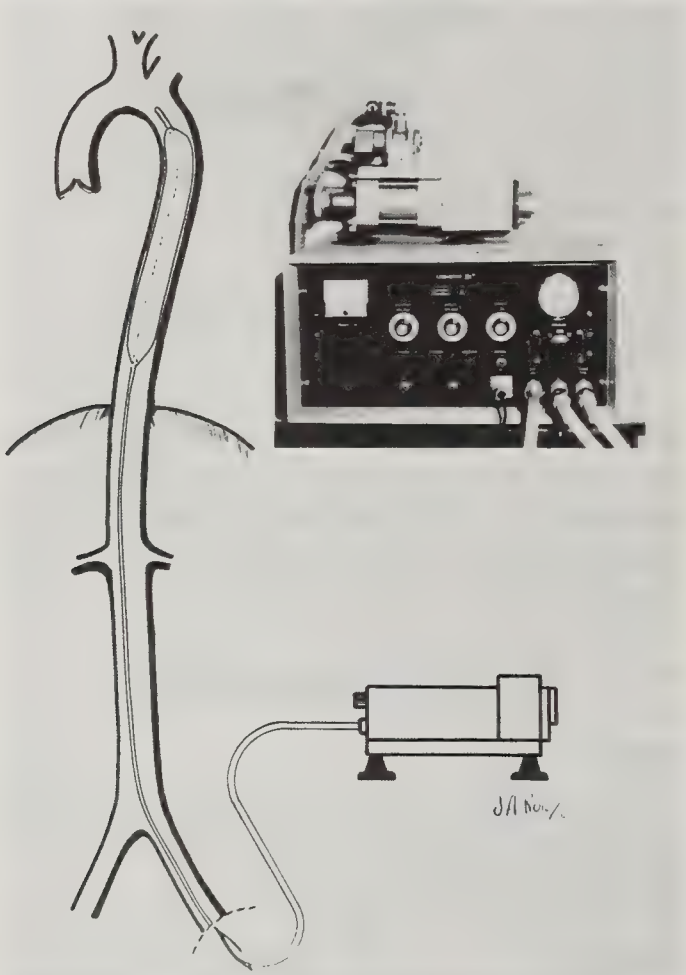


FIGURE 10.—a. Schema for intra-aortic balloon counterpulsation and photograph of the electronic controller connected to the pneumatic actuator and intra-aortic balloon catheter.

Group IV

Six animals were treated by synchronous pulsatile venoarterial bypass with oxygenation (Figure 11). Venous drainage was achieved by cannulating the external jugular veins with a No. 28 Fr. catheter and arterial infusion was through a single No. 24 Fr. femoral cannula. The oxygenator was primed with 1000 cc. of Ringer's lactate solution. Bypass was maintained for four hours following which the animals were observed for an additional hour, then sacrificed.

A pulse pressure generator was specifically developed for this method of assist (Cardiovascular Specialties, Scarborough, Ontario). The generator is a compact device measuring 10 x 10 x 10 cm. consisting of two polycarbonate chambers separated by a silastic diaphragm. The outer contours are convex. The posterior chamber has a central port, which is connected to a pneumatic or hydraulic actuator and an electronic controller. The forward chamber is the pumping chamber in line with the circulation. It has an eccentric ball-valved inlet port which connects with the outflow of an oxygenator and a central valveless port which connects with an arterial catheter. During pump diastole, because of differential resistances in the inflow and outflow lines, withdrawal of the diaphragm results in almost complete filling (96 percent) from the oxygenator (Figure 11). Conversion to arterio-arterial counterpulsation and back to venoarterial bypass can be readily achieved if

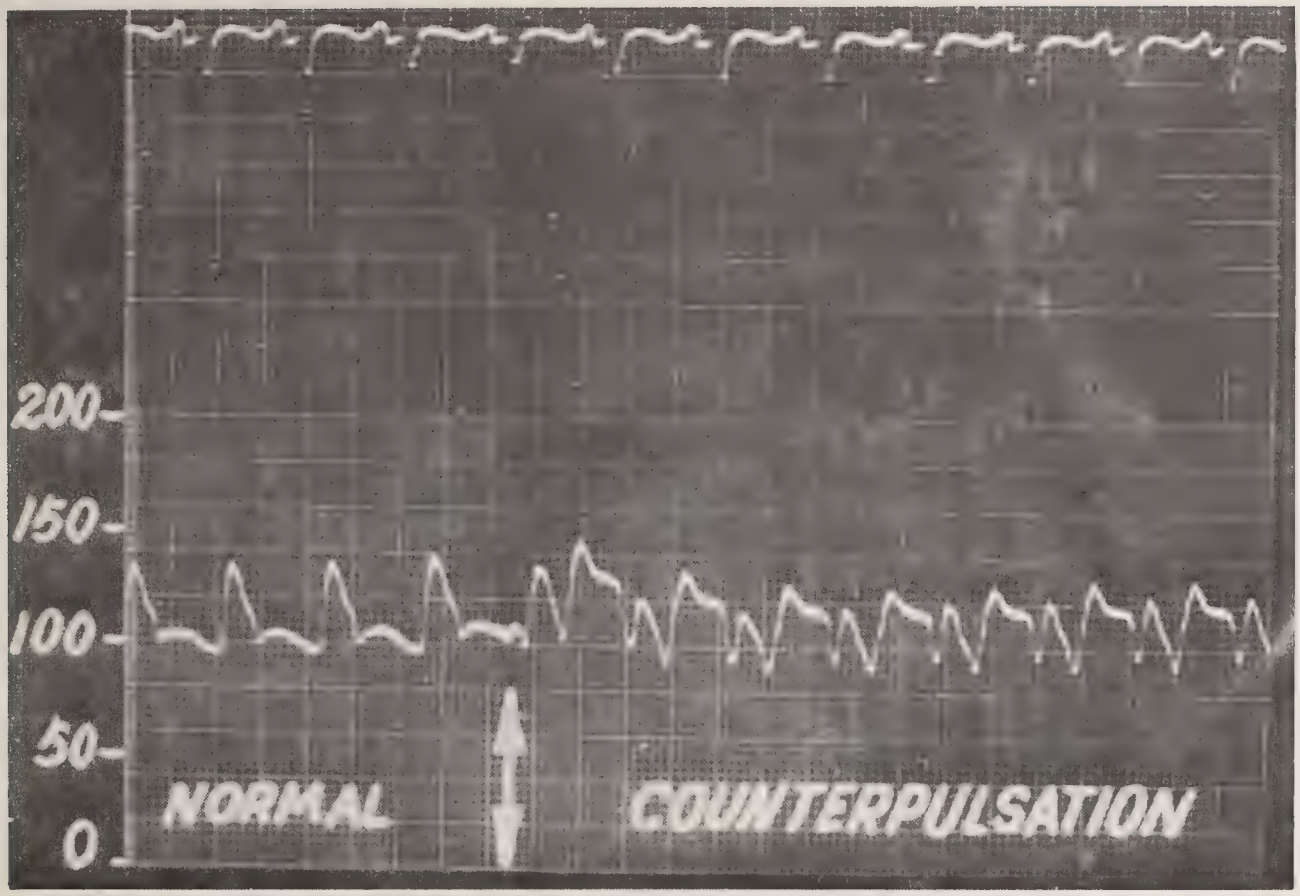


FIGURE 10.—b. Electrocardiographic and aortic pressure tracings prior to and during intra-aortic balloon counterpulsation in a normal dog. Note slight reduction in systolic pressure, but marked augmentation of diastolic pressure.

desired by simply clamping or releasing the pulse pressure generator inflow line. In this experiment, a disposible Temptrol (Bentley Corporation, Norwalk, Connecticut, U.S.A.) oxygenator was incorporated into the system for synchronous pulsatile venoarterial bypass and the pulse generator was hydraulically motivated by a SIMAS controller (Figure 12).

RESULTS

Group I—Untreated Control—Survival

Survival time, once shock was manifest, averaged 72.5 minutes (30–135 minutes). Serial coronary artery ligation produced increasing cyanosis of the anterior surface of the left ventricle and progressive dilation. Death resulted in each instance from ventricular fibrillation. Ventricular fibrillation developed when left atrial pressure averaged 18 mm. Hg. (range 15–22 mm. Hg.). Attempts to resuscitate the heart by cardiac massage and defibrillation were always unsuccessful. The hemodynamic and metabolic findings are graphically illustrated (Figure 13).

Group II—Arterio-Arterial Counterpulsation—Survival

All dogs in cardiogenic shock treated by arterio-arterial counterpulsation survived the experiment, and eight were subsequently sacrificed twenty-four hours later. Two dogs died twenty-two hours post-operatively from atelectasis and hemothorax. At autopsy, no dogs showed evidence of myocardial infarction. The hemodynamic and metabolic findings are indicated in Figure 14.

Group III—Intra-Aortic Balloon Counterpulsation—Survival

All animals survived the four hour treatment period. Hemodynamic and metabolic param-

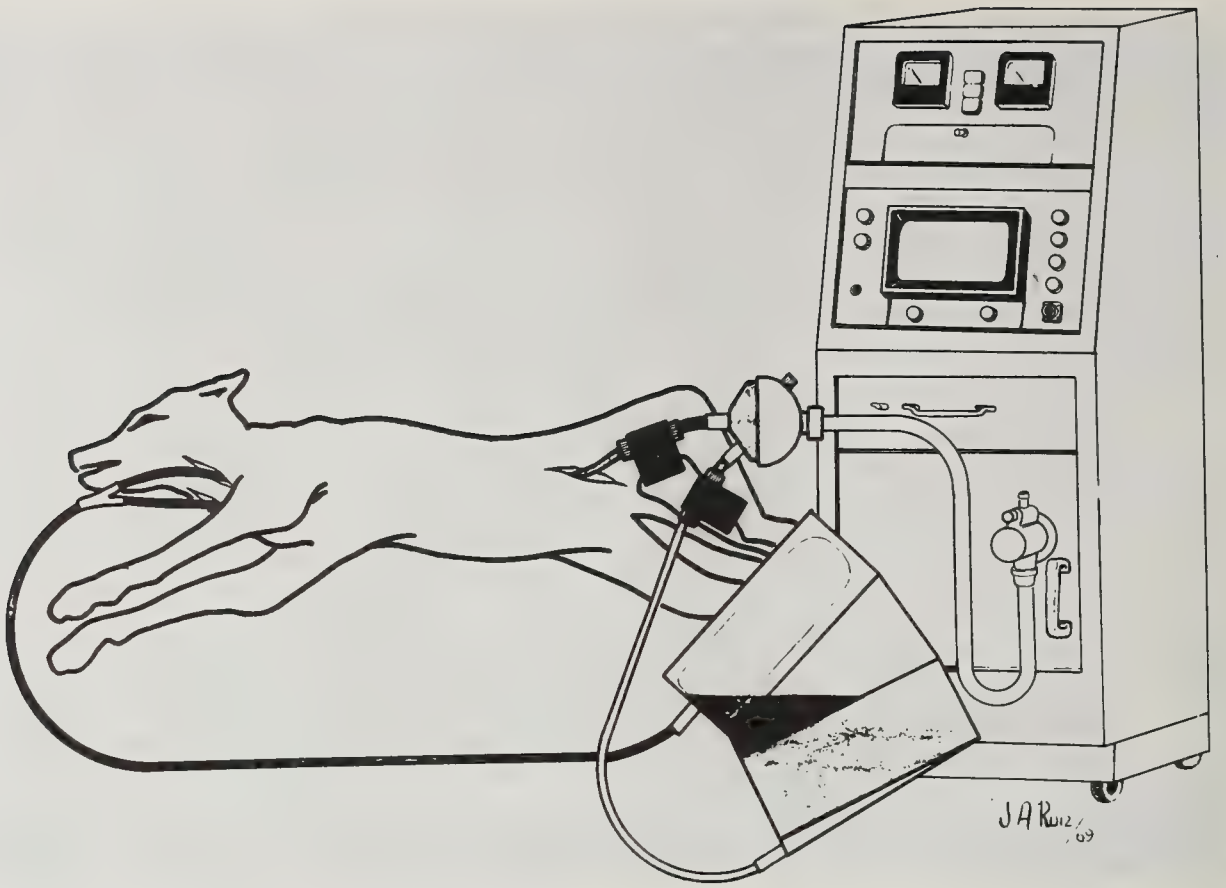


FIGURE 11.—Schema for synchronous pulsatile venoarterial bypass with oxygenation, indicating the sites of cannulation, the disposable oxygenator, the pulse pressure generator and the SIMAS controller.

eters were markedly improved in all dogs (Figure 15). Their condition was good with every likelihood for recovery, had the dogs not been sacrificed one hour after termination of pumping. At autopsy, aortic wall injury was never observed and there was no evidence of embolic phenomena nor organ abnormality.

Group IV—Synchronized Venoarterial Bypass With Oxygenation—Survival

All dogs survived the four hour treatment period. Marked improvement in hemodynamic and metabolic parameters was observed (Figure 16). One hour after cessation of circulatory support they were still in good condition. Autopsy after sacrifice revealed no evidence arterial emboli and histologic studies showed no organ abnormality. Examination of the pulse pressure generator following each experiment also failed to show evidence of fibrin formation or blood clot.

Comments—Study II

The technique of serial ligation of major branches of the anterior descending and circumflex arteries used in this Study produced a heart failure preparation which overcame some of the shortcomings of other methods. It resulted in extensive localized myocardial ischemia and infarction, and a shock state, prior to terminal arrhythmia, long enough to enable evaluation of various support systems. The criteria for cardiogenic shock closely corresponded to those advanced by Friedberg—reduction in venous return, blood volume or cardiac output by 30–40% resulting in clinical manifestation caused by altered hemodynamics and homeostatic changes[14]; by Agress and Associates—acute myocardial infarction proved by electrocardiography or autopsy associated with systolic blood pressure of 80 mm. mercury or less and clinical signs of peripheral circulatory collapse[15] and by Dietzman and co-workers—

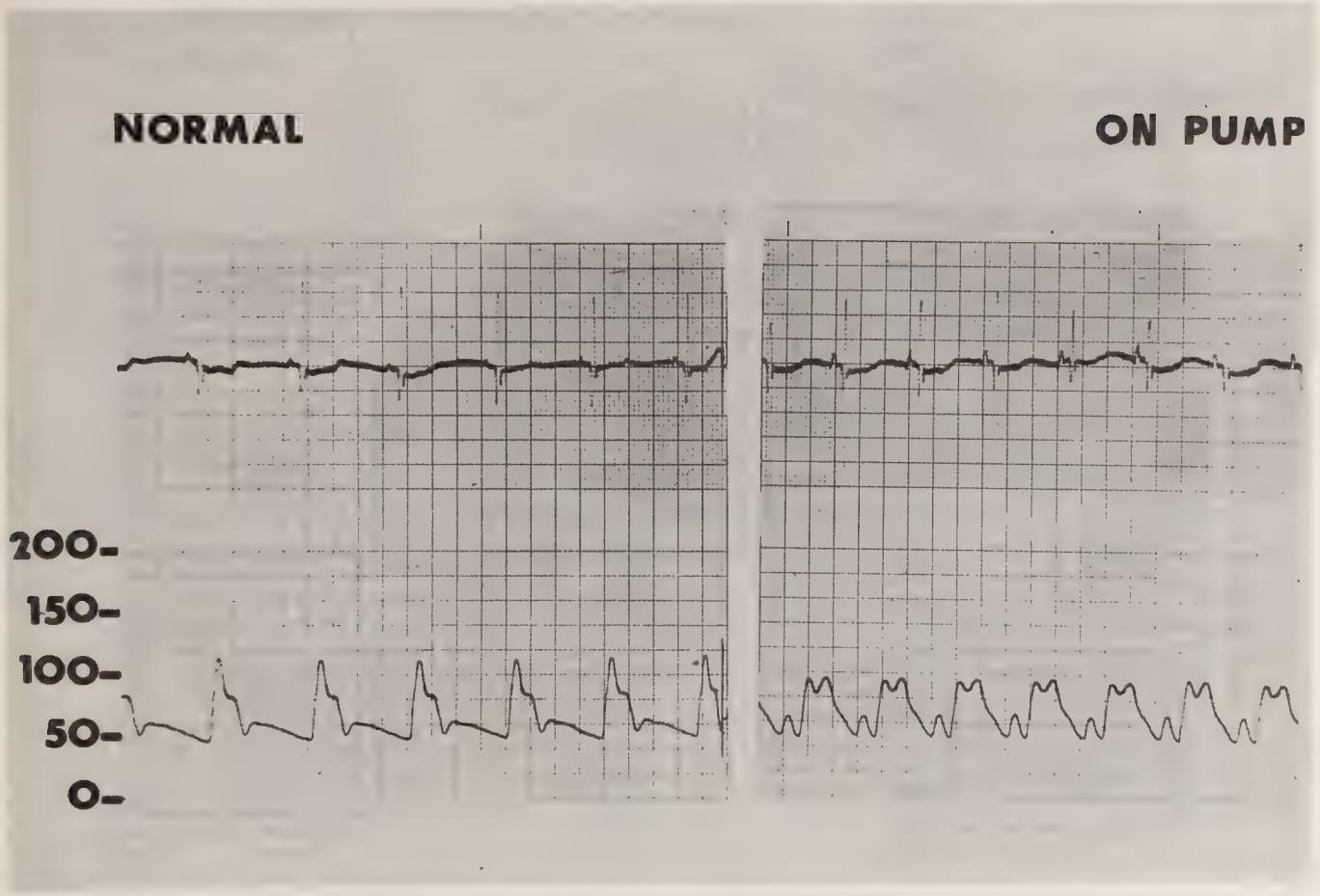


FIGURE 12.—Electrocardiographic and aortic pressure tracings in a normal dog. During synchronized pulsatile venoarterial bypass with oxygenation systolic pressure is reduced and diastolic pressure is markedly elevated.

reduction of pressure volume work of the heart by 50%.

In untreated control animals, myocardial ischemia impaired cardiac function and produced severe lactic acidosis which further aggravated myocardial irritability and contractility. Progressive left heart failure developed and death from ventricular fibrillation ensued. These findings are similar to those reported with other cardiogenic shock preparations. However, contrary to the observations of Agress and Associates, who noted peripheral vasodilation,[15,17] all animals demonstrated increased peripheral vascular resistance when shock developed. Whereas untreated dogs died within one and half hours of onset of the shock state, dogs treated by circulatory support survived the experimental period (irrespective of the mode of therapy). Synchronous circulatory assistance prevented fatal arrhythmia and enabled the animals to survive the ischemic episode.

The results of arterio-arterial counterpulsation are in accord with the findings of Jacoby,[18] Goldfarb,[19] Mantini,[11] and Kishi.[20] Peripheral vascular resistance was diminished and left ventricular work reduced by 39%. Although obviously helpful, it is doubtful whether only one hour of assisted circulation could have by itself produced sustained improvement of circulation following cessation of pumping. The findings suggest that more likely, the explanation was rapid restoration of circulation to ischemic myocardium. The functional significance of increased coronary collateral flow was reflected in arrest of anerobic metabolism. Lactate concentration rapidly diminished. The reduction correlated with marked improvement in cardiac function, return to nearly normal hemodynamics and elevation of arterial oxygen saturation suggesting diminished pulmonary congestion. Since diastolic augmentation must be carried out for a critical period (about thirty minutes in the dog) before increased retrograde col-

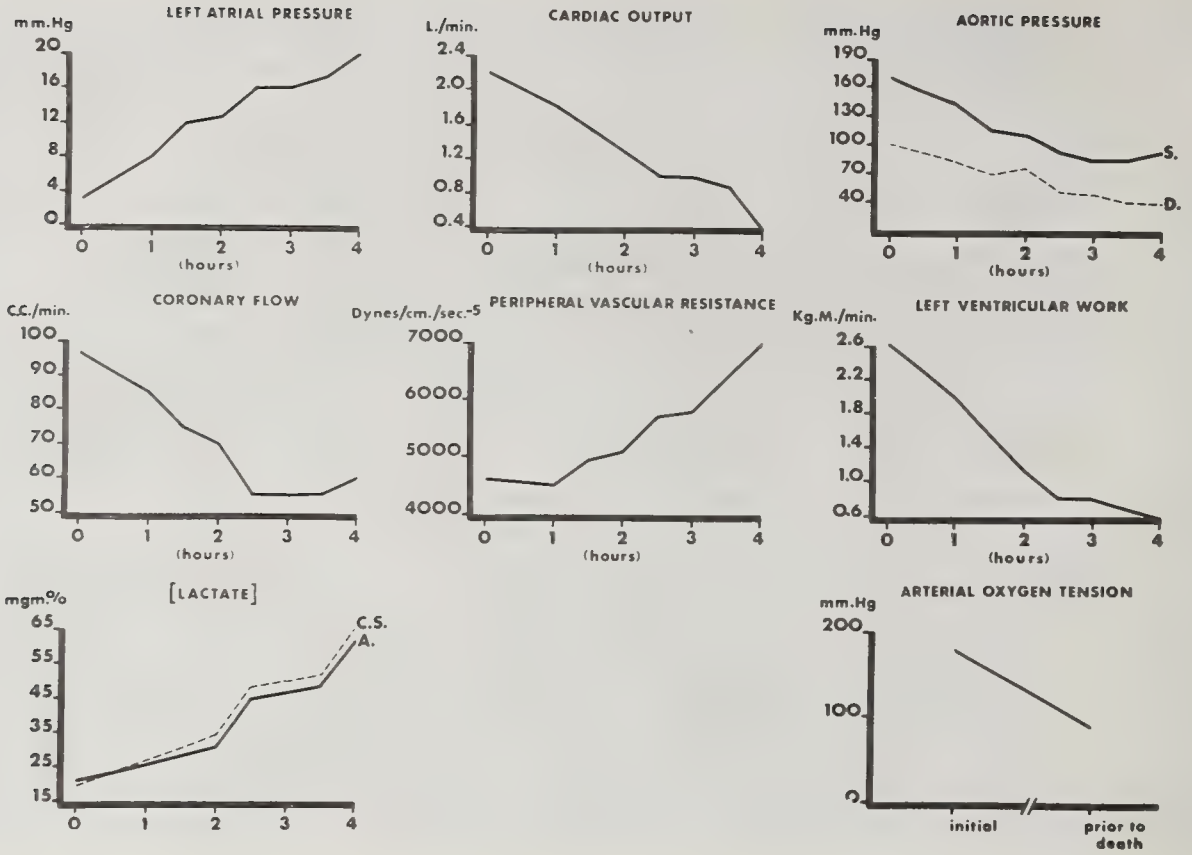


FIGURE 13.—Graphs illustrating mean findings in ten control animals after ligation of left coronary artery branches.

lateral coronary circulation becomes manifest, it appears that counterpulsation also provides the circulatory support required until improved cardiac function becomes apparent and the heart can again maintain normal circulation. The results also suggest that counterpulsation by increasing circulation to ischemic myocardium may have prevented or restricted the extent of myocardial infarction.

Benefits of counterpulsation obviously stem from its three primary hemodynamic effects—reduced aortic systolic pressure, lowered peripheral vascular resistance and enhanced diastolic pressure. The probable mechanism of action in acute cardiogenic shock are schematically indicated in Figures 17A and B. Obvious limitation of arterio-arterial counterpulsation is related to extracorporeal circulation and blood trauma. Other studies show that arterio-arterial counterpulsation can be tolerated for at least four hours with levels of plasma hemoglobin not exceeding 50 mg. percent.[21] In addition, despite original concern about possible peripheral vascular collapse with prolonged periods of counterpulsation, recent experiments have revealed that, provided accurate synchronization is achieved and stroke volume of the pump does not exceed 40–50% of left ventricular stroke volume, no adverse effects develop. Maximal hemodynamic effects were achieved within one hour of initiating circulatory support and coronary as well as peripheral blood flow was always greater during pumping than when the pump was stopped.

In contrast to arterio-arterial counterpulsation, the technical simplicity, stability of response and minimal blood and tissue trauma, make intra-aortic balloon counterpulsation a more attractive modality for circulatory assistance. Although balloon counterpulsation augments dialstolic pressure, it does not lower systolic pressure as strikingly as does arterio-arterial counterpulsation. The need to drastically reduce systolic pressure is questionable, provided coronary and peripheral circulation are adequately maintained. Balloon counterpul-

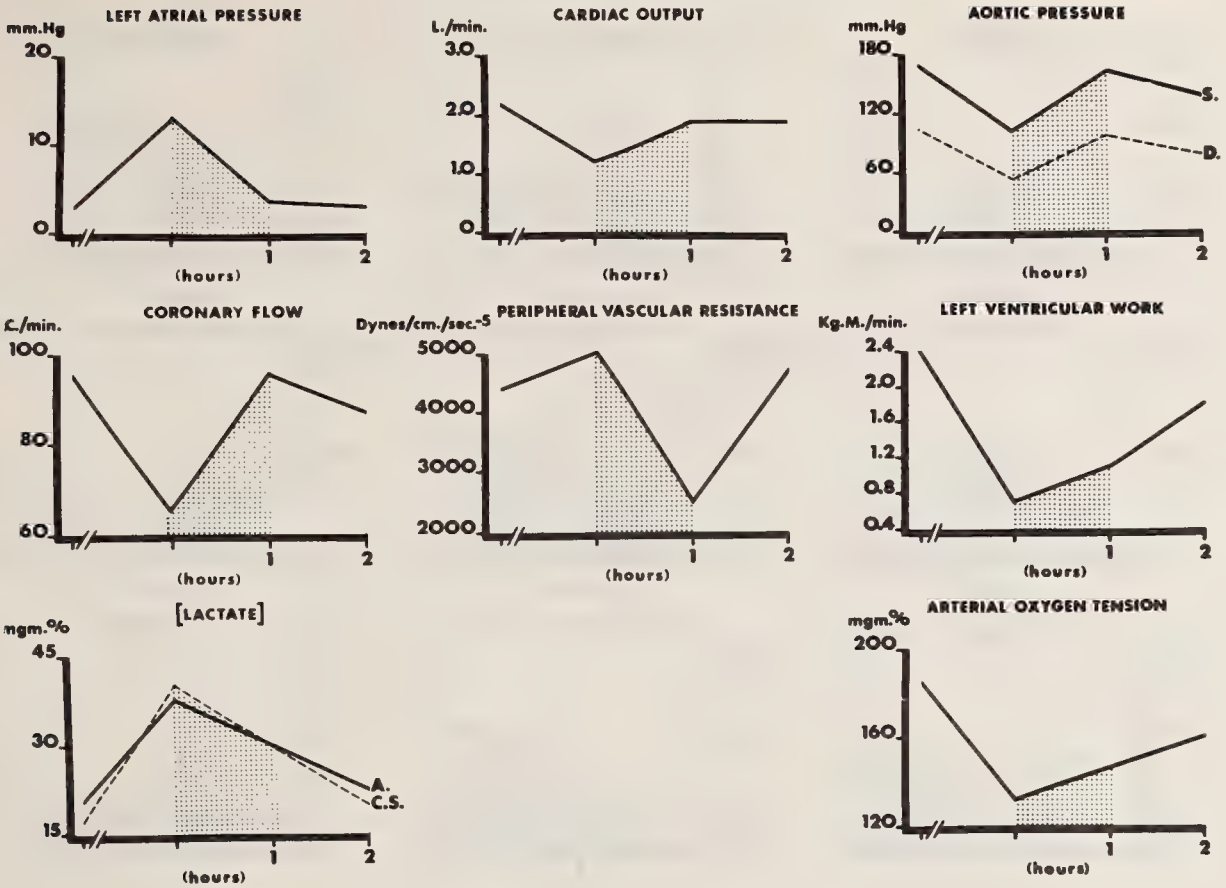


FIGURE 14.—Graphs illustrating the effects on hemodynamics and myocardial metabolism observed in animals treated by arterio-arterial counterpulsation. Mean finding in ten dogs.

sation rapidly restored and effectively maintained peripheral circulation in cardiogenic shock until the heart recovered from its acute ischemic injury. Improvement in myocardial function was undoubtedly related to the increased total and collateral coronary flow as a result of diastolic augmentation and increased cardiac output. The method has obvious advantages over arterio-arterial counterpulsation for it avoids extracorporeal circulation. Balloon rupture and air embolism were not encountered. Recent improvement in design fabrication should minimize this risk.

Pulsatile veno-arterial bypass with oxygenation, like arterio-arterial counterpulsation, has the disadvantage of extracorporeal circulation. However, the primary requirements of any circulatory assistance are fulfilled by the unique characteristics of its pulse pressure generator. It permits the arterial bypass with oxygenation (pumps arterialized blood), incorporates synchronized pulsatile flow and diastolic augmentation (counterpulsation), maintains peripheral circulation, restores normal organ function and is gentle to blood over prolonged periods without evidence of embolization. The latter is probably related to the absence of an outflow valve eliminating a major impediment to flow and a site of turbulence. Use of more nonthrombogenic materials could eliminate the need for anticoagulation and use of a membrane oxygenator could make it safe for extended periods of assistance. The experiments clearly indicated that this method of support was capable of maintaining circulation until the heart recovered from its acute ischemic injury. As with the other methods, the improved myocardial function was no doubt related to increased coronary flow and to enhanced collateral circulation, as a result of elevated mean aortic pressure, particularly increased diastolic pressure. Although diastolic augmentation was not as marked as observed with arterio-arterial counterpulsation, it was nevertheless effective. At low flow states, such as cardiogenic shock, coronary artery pressure in the ischemic bed can approach

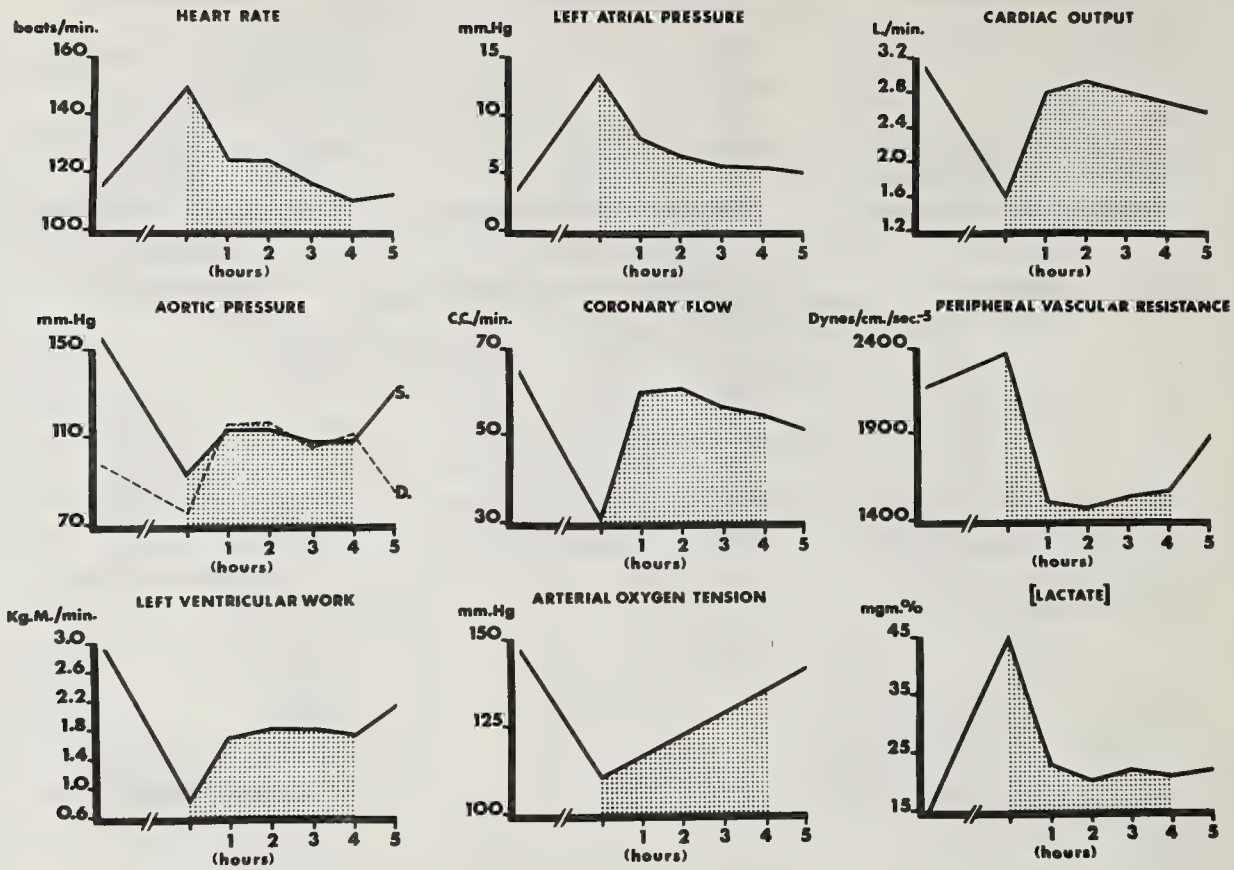


FIGURE 15.—Graphs illustrating the effects upon hemodynamics and metabolism observed in animals treated by intra-aortic balloon counterpulsation. Mean findings in six dogs.

critical closing pressure and therefore increments as little as 10 mm. Hg. can produce a significant rise in coronary flow.

SUMMARY

Chronic coronary insufficiency produced by progressive proximal stenosis by ameroid constrictors results in a reproducible state of significant myocardial ischemia. In the models described, flow in the anterior descending and circumflex coronary arteries decline by 50% within fourteen days, with minimal reduction thereafter, despite continuing proximal stenosis. This preparation vividly demonstrates the natural potential for development of collateral circulation and therefore served as a desirable model for evaluating the effects of counterpulsation. As indicated in the results, retrograde coronary flow could be significantly augmented which correlated with angiographic findings, restoration of myocardial aerobic metabolism, lesser incidence of myocardial infarction and prolonged survival.

The technique of serial ligation of branches of the anterior descending and circumflex coronary arteries resulted in increasingly severe left heart failure ultimately manifesting as cardiogenic shock. There was no significant difference in the time interval between initial ligation and the onset of shock. Untreated, control animals died wthin 135 minutes from progressive left ventricular failure and terminal ventricular fibrillation. This time interval was sufficient for evaluating three different modalities for counterpulsation in treatment groups and determining the advantages and limitations of the various methods for circulatory support.

REFERENCES

1. LITVAK, J. and VINEBERG, A.: Experimental gradual arterial occlusions with *in vitro* and *in vivo* observations, Surg. 46:953, 1959.
2. ROBBINS, S. L., SOLOMON, M. and BENNETT, A.:

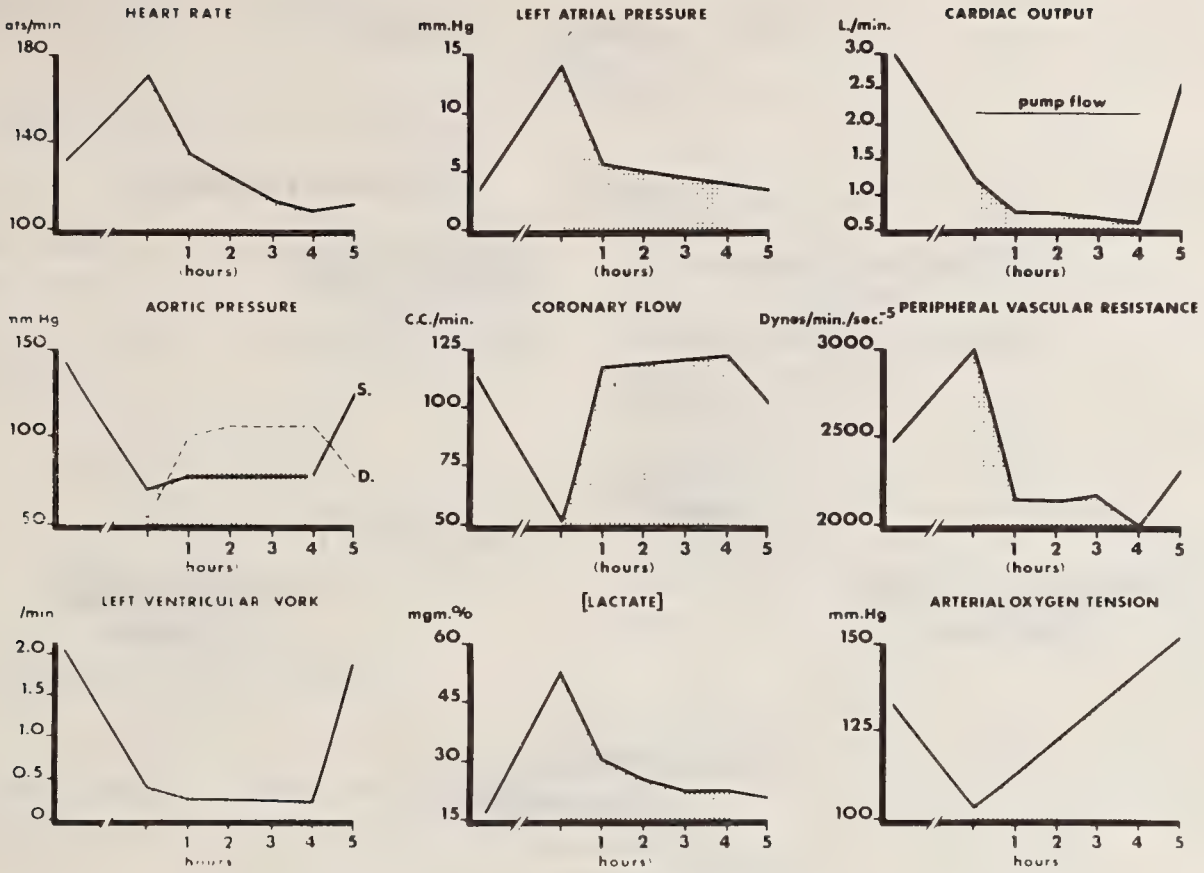


FIGURE 16.—Graphs illustrating the mean effects upon hemodynamics and metabolism observed in animals treated by synchronous pulsatile venoarterial bypass with oxygenation. Mean findings in six dogs.

Demonstration of Intercoronary anastomoses in human hearts with a low viscosity perfusion mass, Circulation 33:733, 1966.

3. HUCKABEE, W. E.: Relationship of pyruvate and lactate in hypoxia, J. Clin. Investig. 37:244, 1958.
4. VANSANT, J. H.: Technique of experimental ligation of the anterior descending coronary artery, Surg. 49:387, 1961.
5. ALLEN, J. B., and LAADT, J. R.: The effects of the level of the ligature on mortality following ligation of the circumflex coronary artery in dogs, Amer. Heart J. 39:273, 1950.
6. BORROMEO, C. P.: The effects of counterpulsation on the development of collateral circulation during acute and chronic coronary artery insufficiency, M.Sc,. Thesis in Experimental Surgery, McGill University, August 18, 1966, pp. 89–90.
7. BURTON, A. C.: Hemodynamics and the Physics of the circulation. In Rush, T.C. and Fulton, J. F.: Medical Physiology and Biophysics (18th ed.), W. B. Saunders Company, Philadelphia, 1960, pp. 659–665.
8. SCHAPER, W. and VANDESTEENE, R.: The rate of growth of interarterial anastomosis in chronic coronary artery occlusion, Life Sci. 6:1673, 1967.
9. ROSENSWEIG, J., BORROMEO, C., CHATTERJEE, S., SHEINER, N., and MAYMAN, A.: Treatment of coronary insufficiency by counterpulsation. An experimental study, Ann. Thorac. Surg. 2:706, 1966.
10. HAHNLOSER, P. B., GALLO, E. and SCHENK, W. G., JR.: The hemodynamics of counterpulsation, J. Thorac. Cardiovasc. Surg. 51:366, 1966.
11. MANTINI, E., KALKE, B., GRANDIN, C., FERLIC, R. and LILLEHEI, C. W.: The use of synchronised counterpulsation and post-extrasystolic potentiation in the treatment of experimental cardiogenic shock, Trans. Amer. Soc. Artif. Intern. Organs 12:57, 1966.
12. SCHAEFER, J., SCHWARZKOPF, H. J., NIEDERMAYER, W., HELD, K. and ULMER, F.: Side effects of diastolic augmentation on renal carotid and femoral blood flow, Circulation 32:2–188: 1965.
13. KANTROWITZ, A., KRAKAUER, J. S., JARON, D., FREED, P., and BUTNER, A. and ROSENBAU, A.: Ventricular support by means of phase-shift balloon pumping: Experimental and clinical experience, Amer. J. Cardiol. 25:107, 1970.
14. FRIEDBERG, C. K.: Cardiogenic shock in acute myocardial infarction, Circulation 23:325, 1961.
15. AGRESS, C. M. and BINDER, M. J.: Cardiogenic Shock, Amer. Heart J. 54:458, 1957.

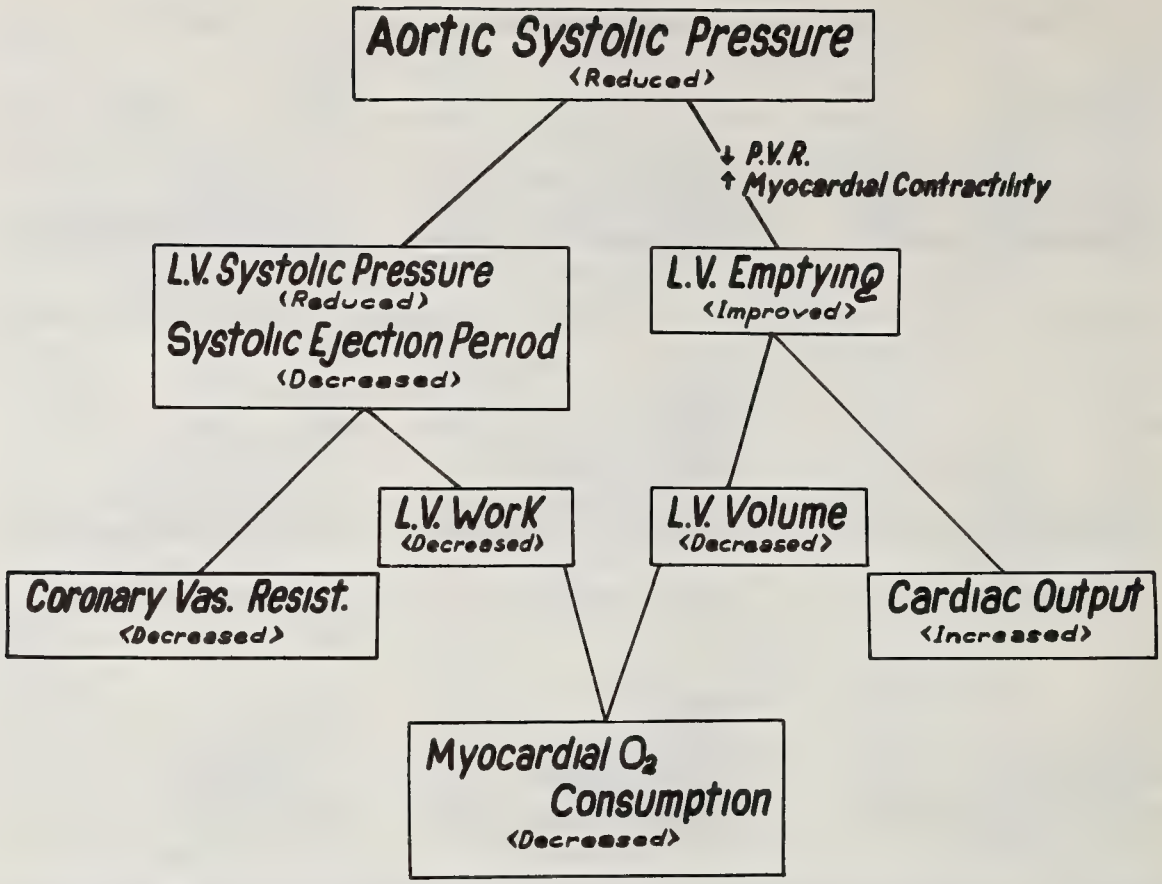


FIGURE 17.—Hemodynamic and metabolic effects of counterpulsation produced by (a) lowering aortic systolic pressure.

16. DIETZMAN, R. H., LYONS, G. W., BLOCH, J. H. and LILLEHEI, R. C.: Relation of Cardiac work to survival in cardiogenic shock in dogs, J.A.M.A. 199:825, 1967.

17. AGRESS, C. M., GLASSNER, H. F., BINDER, M. J., and FIELDS, J.: Hemodynamic measurements in experimental coronary shock: J. App. Physiol. 10:469, 1957.

18. JACOBEY, J. A., TAYLOR, W. J., SMITH, G. T., GORLIN, R. and HARKEN, D. E.: A new therapeutic approach to acute coronary occlusion. II opening of dormant coronary collateral channels by counterpulsation, Amer. J. Cardiol. 11:218, 1963.

19. GOLDFARB, D., CONTI, C. P., BROWN, B. G., and GOTT, V. L.: Treatment of severe cardiogenic shock by diastolic augmentation after ligation and division of the left circumflex coronary artery in dogs, J. Thoracic Cardiovasc. Surg. 51:783, 1966.

20. KISHI, K., OTA, Y., HIRATSUKA, H., AKIMOTO, T., YOKOYAMA, M. and SAKAKIBARA, S.: Mechanical assistance of coronary circulation in the ischemic heart with a newly devised technique, Ann. Thorac. Surg. 9:419, 1970.

21. MERINO, F. and ROSENSWEIG, J.: Unpublished data.

DISCUSSION

CHAIRMAN LA FARGE: Thank you very much, Dr. Chatterjee. I hope that you all got the message: not only is this a beautiful presentation in terms of myocardial insufficiency, coronary insufficiency, but it very nicely demonstrates what happens with counterpulsation when used in almost any form. I think you explained the mechanism so well that it needs no further comment.

PITAMBAR SOMANI, Abbot Laboratories, North Chicago, Ill.: I have a basic reservation concerning dog models for any study of collateral circulation because, as we know well, the dog has preexisting collaterals which we do not find in the human heart or in other primates which have big hearts. Now one of the problems here is that you have preexisting collateral channels and you can easily demonstrate the improved collateral flow. I am very curious to find out if the same thing happens in the human heart or in the other primate hearts.

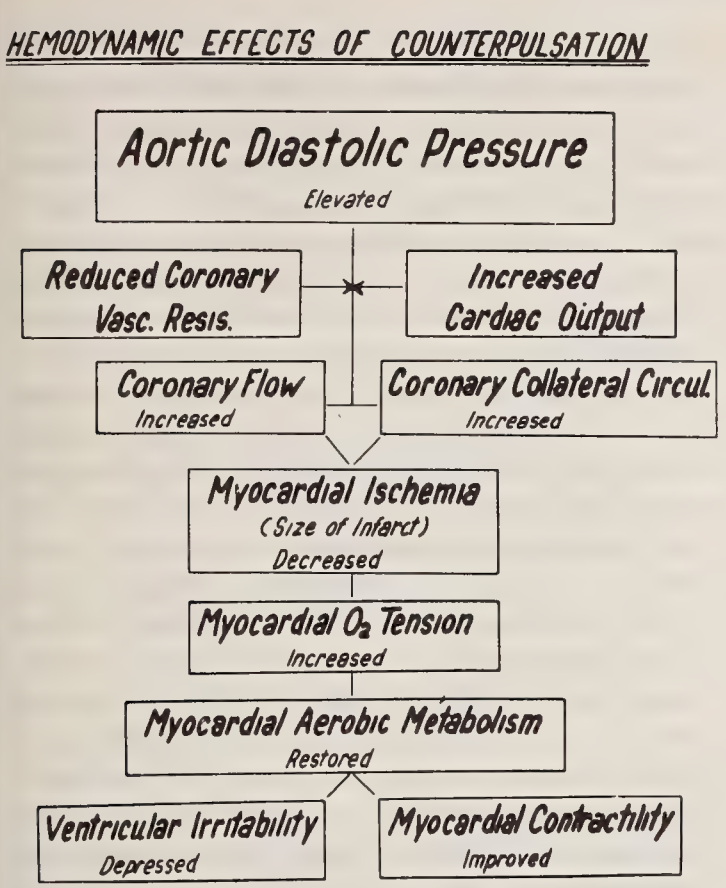


FIGURE 17.—(b) elevating aortic diastolic pressure.

DR. CHATTERJEE: I agree with you that dogs have potential collateral channels, as I have shown in my experiment. As for your question about humans: I do not want to go into the human experiences of counterpulsation. Today we have shown by angiogram that there is improvement in collateral circulation.

DR. SOMANI: I was trying to find out if the same mechanism is operative in humans. The question is: If counterpulsation does increase collateral flow in humans, is this due to the opening of preexisting channels or to some other mechanism?

DR. CHATTERJEE: Counterpulsation probably helps in two ways: First, by increasing diastolic pressure, it improves the coronary artery flow and secondly, at the same time it reduces systolic pressure and the afterload of the heart.

LLOYD G. PHILLIPS, University of Minnesota, Minneapolis, Minn.: Did you have an opportunity to dissect the main trunk of the left coronary artery and thus obviate the effects of the septa branch and also of the left diagonal branch?

DR. CHATTERJEE: Well, we dissected the origins of the anterior descending and the sinistra coronary artery. We did not dissect the left main coronary artery purposely.

DR. M. R. MALINOW, Beaverton, Oregon: I was much impressed by the acute results of counterpulsation. How long did you counterpulsate your dogs in the first study?

DR. CHATTERJEE: In our first study, we counterpulsated for one hour, but in balloon counterpulsation we counterpulsated four hours.

DR. MALINOW: It is a little intriguing to speculate on how one or four hours of counterpulsation can modify the results twenty or thirty days later. Are these studies done under anesthesia? How long does anesthesia last and are the control dogs also anesthetized for the same period of time?

DR. CHATTERJEE: Yes, the experiments are carried out under general anesthesia and the experimental environments for both the control and the treated dogs were more or less the same.

QUESTIONER: Were the dogs anesthetized for the same period of time?

DR. CHATTERJEE: Yes.

E. L. STANLEY, Cox Heart Institute, Kettering, Ohio: Concerning the first study, were aortic pressures measured in the root of the aorta during that short period of time prior to pulsation? I can understand that there is a drop in the pressure with shock, but was there a real drop in aortic pressure in the first study prior to pulsation?

DR. CHATTERJEE: The catheters were passed through the brachial artery to the aortic root in the first study.

DR. STANLEY: My question is: Did you measure the diastolic pressure? Did a decrease indeed occur shortly after placing the ameroid constrictors around the vessels? Was there a drop in aortic root pressure in the first study?

DR. CHATTERJEE: In the first study we did not measure the aortic root pressure in the control dogs. In the counterpulsated dogs we did measure the aortic route pressure.

DR. STANLEY: During pulsation or before pulsation?

DR. CHATTERJEE: Before, after, and during counterpulsation.

DR. STANLEY: What was the pressure during and before pulsation in the aortic route? Do you happen to remember the diastolic pressure? This is quite critical as far as coronary flow is concerned.

DR. CHATTERJEE: Yes. Well I don't remember it exactly right now.

P. N. SAWYER, State University of New York, Downstate Medical Center, N.Y.: As I guess all of you know, Dr. Dennis and a number of his confreres have studied the dog and other animals as cardiogenic shock preparations. We've been asked to attempt to salvage fifty humans using counterpulsation techniques. About twenty different parameters of cardiac output and performance were studied following their going into cardiogenic shock. We found that there is the following angiography in this group of patients: First, by the time a human has gone into cardiogenic shock, he has, in essence, no functioning coronary artery left. Secondly, any support mechanism that we have tested (including partial left heart total bypass, or inter-artery balloon pumping) immediately resulted in an improvement in patients for whom this modality was successfully instituted. In this group of patients and in our combined group study with the cooperation of ten schools, there has still been an 80% mortality and, in our hands, a 95% short-term mortality, and nearly a 100% long-term mortality. This gives rise to the basic conflict of interest here insofar as our experimental animals give us some basic information, but we still do not have an animal preparation that corresponds in any way to the human preparation. I believe this leads to another obvious comment. At this point, I think almost everybody has reached the conclusion, as has Dr. LaFarge, that some form of permanent left ventricular replacement or support should take the load permanently off from the essentially non-functioning human left ventricle. This will be necessary to save the majority of human patients whom we see in cardiogenic shock.

DR. CHATTERJEE: I don't wish to comment.

CHAIRMAN: I would like to comment on this since Dr. Chatterjee does not wish to. I call your attention to an article, which I cannot cite exactly for you, by Alexander Leaf at the Massachusetts General Hospital, that appeared in the *American Journal of Medicine* last year and is concerned with the mechanism of cell death due to ischemia and edema. It discusses the mechanism that you suggested, i.e., the concept of a critical opening pressure in a vessel which is occluded both by the disease process and by the edema which results in transient ischemia following that process. It is comparable to some sort of self-perpetuating decreased coronary flow. It has also been clinically observed at the Massachusetts General Hospital where the Kantrowitz or rather the Avco balloon counterpulsation was repeatedly used (and is used now in a number of patients) that the same phenomenon occurs, although I don't know the effect on coronary collateral circulation in humans. It is certainly evident that the same kind of long-term unexpected and unexplained benefit occurs in terms of decreased lactate production and increased coronary flow. It certainly results in clinical improvement, i.e., restoration of what one would consider to be clinical parameters of recovery. Now this is not always obtained, but it suggests that at least the critical opening pressure reoxygenation of areas which are in borderline ischemia and suffering thereby, together with a sort of self-perpetuating process, may indeed be part of the mechanism. How this affects coronary collateral circulation has yet to be seen.

DR. SAWYER: I concede that, but why do 80% of them die? That is, I concede, since that is exactly what we have found. They all display improved lactate metabolism, they all display improved cardiac output, they all display improved systolic pressures, they all have effective increased workloads and decreased diastolic pressures and they all die.

CHAIRMAN LA FARGE: Well, that is true. They don't *all* die, but a very significant percentage are assisted for a very short period of time and this is why there is the considerable interest in using this technique. First of all, it gives you the time to deal with a patient—time which was not available previously. Secondly, in that period of time not only standard or newer methods of drug treatment can be applied, but coronary angiography can be done as well, and the differential diagnosis between small vessel dis-

ease and large obstructions can be made. Subsequently, it is possible to go on to something definitive like saphenous vein bypass which was done several times at Massachusetts General Hospital. I'm still selling myself down the drain because I believe, as you do, that there will be a very significant number of those who will go on in spite of these efforts to develop further cardiogenic shock and cannot be bailed out; that is where my interest in long-term assistance arises. Would you like to comment?

DR. CHATTERJEE: Yes. I'd like to comment on our experience in counterpulsation. We have done arterial counterpulsation in twelve cases, out of which four patients have survived. The longest period of survival is four years.

DR. SAWYER: What parameter was used to determine whether the patient was in cardiogenic shock?

DR. CHATTERJEE: No palpable pressure and low cardiac output—complete clinical signs of cardiogenic shock. We have also treated nine cases by balloon pumping and salvaged five patients; one of these died after six months. So there are four living patients.

DR. SAWYER: We have seen a number of patients considered to be in cardiogenic shock; they were salvaged by pharmacotherapy and it was found, in each instance when they were angiogrammed, that they had at most one severe coronary arterial occlusion. All those patients who died had autopsies done on their coronary arteries, and they were all found to have very severe coronary arterial disease; they were all found to have a very acute or a very poor collateral formation; they were all found to have essentially no patent proximal coronary artery—a very severe three vessel disease. I maintain that the reason why we are all here is because everybody here concedes that the majority of these patients are going to need one of three things done. (1) They must have revascularization of what's left of their myocardia; (2) they must have the existing dead myocardia removed so that, hopefully, enough left ventricle is left to act as an effective pump; or (3) as Dr. LaFarge has now maintained on the basis of his own actions for the past ten years, they should have either an auxiliary or a replacement pump.

CHAIRMAN: I am not trying to say that counterpulsation is the ultimate answer, but it probably can be used as a temporary support in many of these cases and probably in some patients who do not require it.

DR. SAWYER: We agree.

CHAIRMAN: I'd like to conclude on that note also, i.e., that we need both types of support, as you will see over the years. We need the parallel development of both, including the parallel development of transplants.

PULMONARY ARTERY CONSTRICTION IN THE CAT: A MODEL FOR VENTRICULAR HYPERTROPHY AND CONGESTIVE HEART FAILURE

J. F. Spann, Jr.* and G. M. Lemole**

The cat offers an excellent model to study hypertrophy and congestive heart failure. By banding the pulmonary artery and allowing hypertrophy and/or congestive heart failure to occur, it is possible to accurately measure and record the changes in contractility by measuring the force velocity, relationship, V_{max} and length-tension curves. Because of the unique anatomy of the papillary muscle of the right ventricle of the cat, accurate in vitro measurements can be made in the papillary muscle bath. These can be compared to in vivo intact ventricle preparations and the hemodynamical alterations studied and analyzed in light of the in vitro measurements. This method also allows us to obtain the tissue for biochemical analysis in order to measure substrate availability, energy storage, energy utilization and conversion, and excitation-contraction coupling. Lastly, we can record the "effects of inotropic intervention by use of paired electrical stimulation, changes in frequency of contraction and the addition of strophanthidin and norepinephrine in the normal, hypertrophied and failing heart."

INTRODUCTION

Ventricular hypertrophy and congestive heart failure have deen induced in dogs,[1] guinea pigs[2] and rabbits[3] and such experimental models have provided considerable insight into these pathologic states. However, a quantitative description of the contractile state of the cardiac muscle isolated from the hypertrophied or failing heart has not been available. The cat offers unique advantages for analyzing the contractile state of the myocardium in hypertrophy with and without congestive heart failure. This animal is suitable for hemodynamic studies, measurement of the contractile performance of the intact ventricle,[4] and the papillary muscle from the right ventricle is small enough to permit in vitro oxygenation and its fibers are oriented in parallel and thus, allow quantitative definition of the heart muscle function per unit of muscle mass. Further, the functional characteristics of the cat papillary muscle preparation have been described in detail[5] and have sufficient uniformity to allow a meaningful comparison of the myocardial contractile state of one group of cats to that of another group. To provide the animal model for such studies, a method of production of right ventricular hypertrophy with and without congestive heart failure in the cat was developed. This technique imposes a chronic pressure load on the right ventricle by reduction of the lumen of the supravalvular portion of the main pulmonary artery with a constricting clip.

* Chief, Section of Cardiology, Program Manager, Cardiopulmonary Program, Professor of Medicine, Temple University Health Sciences center.

** Chief, Section of Cardiac and Thoracic Surgery, Associate Professor of Surgery, Temple University Health Sciences Center.

BACKGROUND

The pathophysiology which attends both cardiac hypertrophy and congestive heart failure has been a subject of intense interest and investigation for many years. Considerable attention has been focused on the clinical manifestations of heart failure, the hemodynamic alterations of the failing heart as a pump, and the deleterious effects of heart failure on other organ systems such as the kidney. More recent investigations have emphasized an understanding of the hypertrophied and failing heart as abnormal cardiac muscle with secondary emphasis on the function of the heart as a pump.[6] It has now become quite apparent that considerable depression of intrinsic myocardial function may be present with no apparent alteration in hemodynamic function: elevation of ventricular end-diastolic pressure, increased arteriovenous oxygen differences, and decreased cardiac output are frequently late manifestations of failing myocardial function or, indeed, can be due to extra myocardial factors. For example, an increase in end-diastolic pressure is not a reliable

indication of heart failure since alterations in the passive pressure-volume relationship of the ventricular chambers will alter end-diastolic pressure at any ventricular volume. Thus, the importance of accurately assessing the contractility of the myocardium in the study of the patient with heart disease has become apparent; it is imperative to evaluate the myocardial contractility in both experimental and clinical ventricular hypertrophy and congestive heart failure to achieve a proper basic understanding of these conditions. Further, the compensatory mechanisms which maintains circulatory function when cardiac muscle function is depressed are more readily understood when congestive heart failure is considered in terms of cardiac muscle work, load and performance.

The detailed work of A. V. Hill on skeletal muscle has been extended recently to the understanding of the intrinsic contractile state of cardiac muscle, making it possible to examine the function of the heart in terms of myocardial muscle mechanics.[5] Cardiac muscle is thought of as a three-component mechanical model with a freely extensible contractile element which when activated develops force and shortens. This contractile element is connected in series with an elastic component, the series elastic and in parallel with another elastic component referred to as the parallel elastic. Myocardial contractile state is a basic property of the contractile element. Study of the force velocity relationships of the isolated heart muscle with extrapolation to zero load allows determination of the maximum velocity of shortening and a quantitative description of the mechanical function of the contractile element. Cardiac muscle function can be described in terms of the instantaneous force, velocity and shortening and the function of the intact ventricle can also be expressed within these concepts.

To more clearly delineate the pathophysiologic process, several questions had to be answered. What is the degree of hypertrophy and the time course of hypertrophy of the ventricle after an excessive load was placed on the heart? What is the intrinsic contractile state of each unit of myocardium in the hypertrophied heart with and without overt heart failure? Is overall compensation in the hypertrophied heart maintained by an increased muscle mass, each unit of which has a normal or even increased contractile function or is the function of each unit of hypertrophied muscle depressed while overall compensation is maintained by an increase in the contractile mass? What is the contractile state of each unit of myocardium in the hypertrophied ventricle when overt heart failure has occurred? Does failure of the ventricle as a pump occur in the presence of inadequate contractile mass although the contractile function of each unit is normal or even supernormal, or is congestive heart failure the consequence of the depression of the contractility of myocardium that is not compensated for by the increase in muscle mass?

Furthermore, elucidation of the biochemical basis for congestive heart failure is necessary. Of particular interest is the efficiency of the conversion of chemical energy to mechanical work in heart failure. Adenosine triphosphate (ATP) and creatine phosphate are the immediate sources of energy for muscle contraction and it has recently been demonstrated that the utilization of these high energy phosphates by isolated heart muscle preparations can be directly correlated with their mechanical activity.[7,8] This has been accomplished by inhibiting energy production by blockade of both oxidative phosphorylation and anaerobic glycolysis, and subsequently estimating the utilization of energy stores both at rest and during activity.

Lastly, although the synthesis of myofibrillar protein in heart subjected to a systolic overload has been studied, the extent to which the connective tissue elements participate in this response has not been defined. In hypertrophy of skeletal and smooth muscle the content of collagen has been shown to increase along with muscle proteins without a change in their relative proportions.[9,10] In the normal human heart, the concentration of collagen is independent of age, except for increase in the right ventricle during the first ten years of life. Blumgard et al.[11] measured collagen concentrations in the hypertrophied human heart and found it increased in nine of twenty-four. Of these nine, five had demonstrable coronary disease. However, Montforte and Perez-Tamaya[12] found

that the ratio to collagen to muscle protein in human left ventricles was unaltered in various types of heart disease accompanied by myocardial hypertrophy while Oakin and Bouecek[13] found an increase in the left ventricular collagen content in nearly all of twenty-two hearts with significant hypertrophy. Since connective tissue contributes to ventricular compliance and since increases in its concentration might interfere with contractile activity, it is of significance to determine the changes in connective tissue which accompany hypertrophy in an experimentally controlled setting, in the absence of such potentially modifying factors as coronary disease and ischemia. Since hydroxyproline is found only in collagen and forms a constant portion of collagen, the concentration of collagen and thus, the connecting tissue components in the myocardium can be determined by measuring hydroxyproline.

TECHNIQUE

The pulmonary artery of the cat is constricted by a circular clip which is applied in the following manner. Animals are anesthetized with intravenous sodium methahexatol 15 mgm./kg. To permit endotracheal intubation and control of respiration, intravenous succinylcholine lmg/kg. is then given and intermittent positive pressure ventilation is applied by a Harvard respirator using air. Under sterile conditions, the chest is opened through the anterior one third of that left intercostal space which lies 2 cm. cephalad from the apical impulse; the pericardium is widely excised and a 5 mm. segment of main pulmonary artery just distal to the pulmonic valve is dissected free of the ascending aorta. This segment of the pulmonary artery is cleaned of adipose and connective tissue and a curved hemostat passed from left to right behind the pulmonary artery so that its tip is exposed between this vessel and the aorta. One free end of the suture threaded thru the tubing of the clip (Figure 1) is then picked up and pulled around the pulmonary artery as the hemostat is removed. The clip is then positioned posterior to the pulmonary artery and this vessel is insinuated thru the opening in the circle into the clip. The ends of the clip are approximated by slight tension on the two ends of the suture which are then tied together. After the clip is in place, the respiratory gas mixture is changed to 95% oxygen and 5% carbon dioxide, the incision closed, the endotracheal tube removed, and the animal allowed to recover for 30 minutes in a chamber containing 95% and 5% carbon dioxide.

The clips are made in the following manner. PE-50 polyethylene tubing is wound in a spiral around a rod with a diameter equal to that desired as the internal diameter of the clip. A single cut is then made through the spiral tubing in the long axis of the rod so as to result in several pieces of tubing of appropriate length. Copper wire of 0.016 inch diameter and a 40 cm. length of 00 silk suture are inserted into each segment of tubing and the wire is cut to correspond to the length of the polyethylene. The tubing, wire and suture are bent around the rod until the cut ends of the tubing are brought together, thus, forming a circle with the shape maintained by the stiffness of the wire. Clips with an internal diameter of either 2.8 or 3.5 mm. are used in the adult cat weighing between 1.8 and 2.4 kilograms.

Up to 90 days after the pulmonary banding procedure, right heart catheterization is carried out under light intravenous Nembutal anesthesia, 20 mg./kgm. A cannula is placed in the

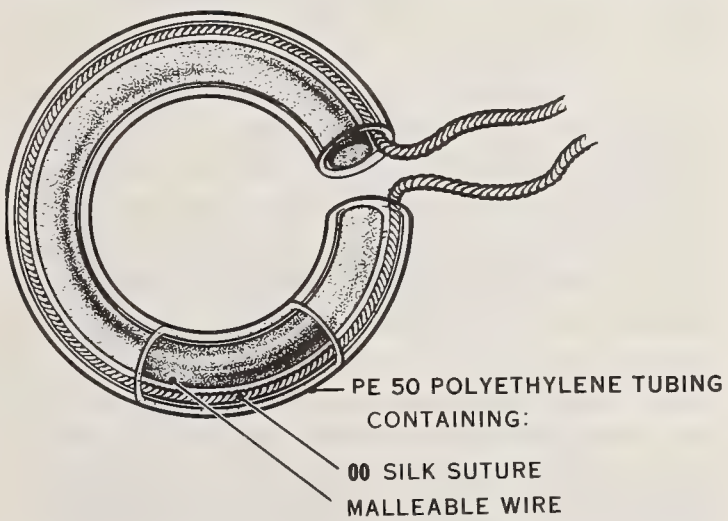


FIGURE 1.—Clip used to constrict the pulmonary artery. It consists of malleable wire and silk suture within polyethylene tubing. The slot which interrupts the circumference of the clip is 0.5 mm. wide. (Reproduced with permission from *Proceedings of the Society for Experimental Biology and Medicine* 125:523, 1967.)

descending aorta via the femoral artery, the electrocardiogram is monitored and a number five French birdseye catheter is placed in the body of the right ventricle via the right external jugular vein. Right ventricular and aortic pressures are measured by Statham force transducers (P23Db) and all signals are recorded on a multichannel Sanborn oscillograph. The zero reference point is taken as the midchest position, corresponding to the level of the right atrium. Cardiac output is determined by the dye dilution technique with the injection of 1.25 mg. indocyanine green (1 cc.) into the right ventricle and sampling from the abdomonial aorta. Mixed venous and arterial blood samples were obtained from the right ventricle and aorta, respectively, and analyzed for oxygen content by the Van Slyke technique.[14]

RESULTS

Hypertrophy and Failure

By using this method, the pulmonary artery can be constricted to an average residual lumen of 10% of normal by the 2.8 mm. clips and to 20% of normal by 3.5 mm. clips. Pulmonary banding by either clip produces right ventricular systolic hypertension and results in right ventricular hypertrophy. The increment increase in weight of the right ventricular free wall was 80% of the control weight or greater in every animal from either group studied one week or longer after constriction of the pulmonary artery. Following constriction with 2.8 mm. clips overt congestive heart failure was present in addition to right ventricular hypertrophy in 80% of the cats sacrificed an average of 26 days after operation. Criteria used to denote overt congestive heart failure included pleural fluid, hepatic congestion, ascites, elevation of right ventricular end-diastolic pressure (Figure 2) reduction of cardiac index and an increase in the arteriovenous oxygen difference. Gross pleural and/or ascitic fluid were present in 75% of the animals with heart failure; in 75% of these animals, two or more of the criteria of failure were present. The cats with the 2.8 mm. clips also had marked dilatation of the right ventricular chamber and some demonstrated right ventricular pulsus alternans. Sixty percent of the animals with 2.8 mm. clips died with evidence of severe congestive heart failure before catheterization or sacrifice for study.

Right ventricular weight increased promptly after the constricting operation and then rose less rapidly (Figure 3). The right ventricular weight in normal cats averaged 0.55 ± 0.02 (S. E. M.) gms./kgm. body weight. These weights were significantly increased above normal one or two days after constriction, averaging 0.7 ± 0.04 and 0.9 ± 0.05 kilograms, respectively. In cats in which right ventricular hypertrophy alone was produced by constriction of the pulmonary artery to 20% of its original lumen with a 3.5 mm. internal diameter clip, the right ventricular weight averaged 1.05 ± 0.6 gms./kgm. In animals in which congestive heart failure, as well as right ventricular hypertrophy was produced by constriction of the pulmonary artery to 10% of its original lumen by a 2.8 mm. clip, the right ventricular weight averaged 1.33 ± 0.10 gms./kgms. Similar results were observed in the left ventricle of guinea pigs after constriction of the ascending aorta.[15]

The very rapid onset of significant ventricular hypertrophy after an abnormal load was imposed on the heart is of interest and somewhat

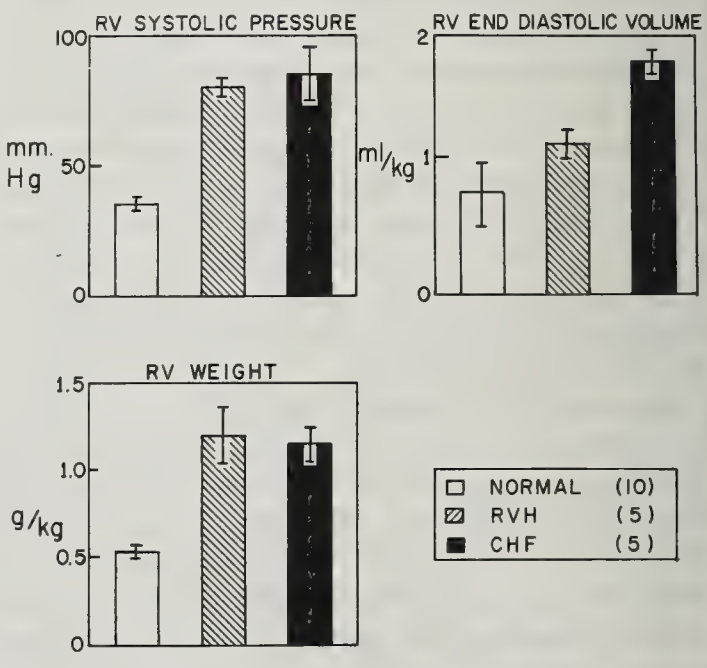


FIGURE 2.—Right ventricular (RV) systolic pressure, right ventricular end-diastolic pressure (RVEDP) and right ventricular weight from normal, hypertrophied and failing cats.

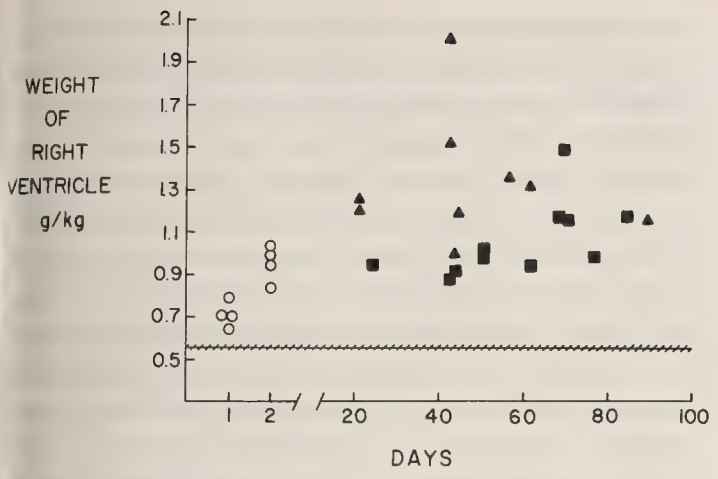


FIGURE 3.—Relation between right ventricular weight and time at which pulmonary artery was banded. Average normal right ventricular weight and standard error are represented by the sawtooth line. Circles and triangles = animals with a 2.8-mm. clip. Squares = animals with a 3.5-mm. clip. The abscissa represents days after pulmonary artery was constricted. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* 21:341, 1967.)

surprising. The wet weight to dry weight ratio is the same in the normal and hypertrophied groups of heart muscles 48 hours after contriction of the pulmonary artery, thus, excluding the possibility that the increase in ventricular weight is due to excessive tissue water. Myocardial RNA synthesis, as measured by RNA polymerase activity, has also been shown to be increased within 24 hours after an excessive work load was imposed on the left ventricle.[16] Some evidence is available to suggest that ventricular hypertrophy can occur with similar rapidity in man. Thus, the data reinforces the concept that when the heart is faced with an abnormal load, there is a rapid increase in the contractile element mass.

Contractility

Next to be considered is the contractile function of the myocardial units within this increased contractile mass. The following discussion is based on data from an experimental plan which compared, under controlled conditions, the function of isolated papillary muscles obtained from the right ventricles of three groups of cats: (a) normal cats; (b) cats with pulmonic constriction and right ventricular hypertrophy, but without congestive heart failure; (c) cats with pulmonic constriction in which, despite the development of right ventricular hypertrophy, heart failure developed. All muscles were studied under the same condition in a myograph in oxygenated Kreb's solution. This system allowed measurement of the force, muscle length and first derivative of these variables.[17] Length-tension relations, the isometric contraction, force velocity relations and the response to inotropic interventions also were determined.

The length-tension relation is qualitatively similar but quantitatively quite different in muscles from the three groups of animals (Figure 4). The actively developed tension is less than normal at all muscle lengths along the length-tension curve in preparations from animals with right ventricular hypertrophy and congestive heart failure. Maximal active tension in the muscles from the cats with hypertrophy is reduced, averaging 5.5 ± 0.6 gms./mm. sq. compared with the normal value of 6.2 ± 0.7 gms./mm. sq. whereas, the maximal isometric tension of the muscles from cats with conges-

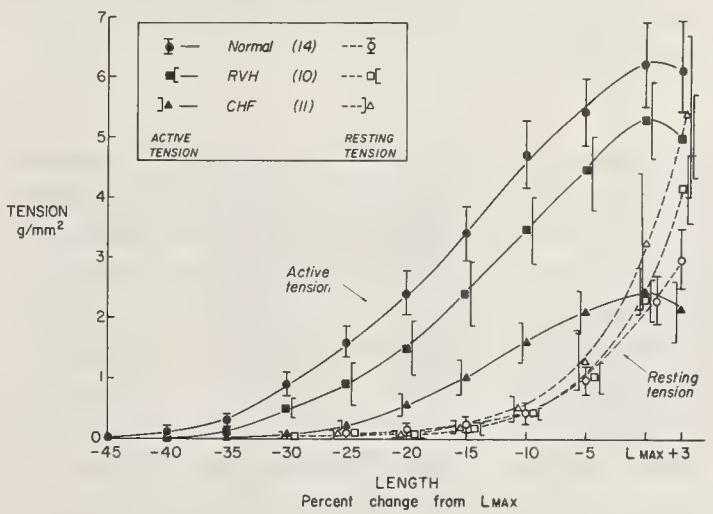


FIGURE 4.—Relation between muscle length and tension of the papillary muscles from normal (circles), hypertrophied (squares) and failing (triangles) right ventricles. Open symbols = resting tension; solid symbols = actively developed tension. Each value is the average of the group; vertical lines with cross bars = ± 1 S.E.M. Tension is corrected for cross-sectional area (gm./mm.^{2}). Numbers in parentheses = number of animals. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* (21:341, 1967.)

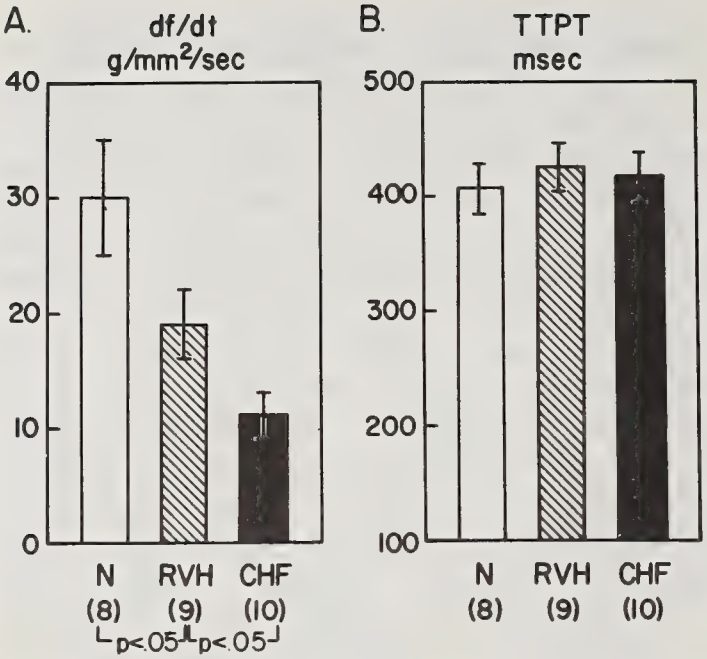


FIGURE 5.—Left, maximum rate of isometric force development shown for the three groups of muscles in g/mm²/sec. All muscles were studied at the apex of their length-tension curves. Right, time from stimulation to the peak of developed isometric tension in milliseconds. All muscles were studied at the apex of their length-tension curves. Numbers in parentheses = number of animals. Vertical lines with cross bars = ±1SEM. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* 21:341, 1967.)

tive heart failure is markedly reduced, averaging 2.4 ± 0.4 gms./mm. sq. On the other hand, as shown by the dotted lines on the lower portion of Figure 4, there are no significant differences among the average resting length-tension curves in any of the three groups of muscles. These observations allowed elimination of the possibility that the decrease in force in failing muscle is due to the disengagement of the actin and myosin filaments known to occur on the descending limb of the Frank-Starling curve. Specifically, it was noted that the tension at all points on the ascending limb of the active length-tension curve is depressed in the failing muscle. The decreased tension developed by the failing and hypertrophied heart muscle can be related to an intrinsic weakness of the muscle rather than an abnormal position on a basically normal length-tension curve, thus allowing rejection of the hypothesis that the decreased contractility of the failing heart muscle is due to its operation along the descending limb of the Frank-Starling curve. Also, the depression of the active length-tension curves could not be attributed to a change in compliance since the resting length-tension curves of the hypertrophied and failing heart muscle were normal.

The time relations of the isometrically contracting muscle can also be determined in the right ventricular papillary muscle preparations. The rate of tension development (dF/dt) and the time required for development of peak tension were determined (Figure 5). The dF/dt is markedly depressed in the hypertrophied and failing heart muscle. However, the time to peak tension is normal in both of these groups of muscles. Since series elasticity is unchanged in cardiac muscle from the hypertrophied and failing heart,[18] the depression of dF/dt reflects a decrease in the intrinsic speed of contraction and is a manifestation of a reduction of the intensity of the active state in the failing heart muscle. However, the time to peak tension is not altered. This later finding suggests that, although the maximal intensity of the active state

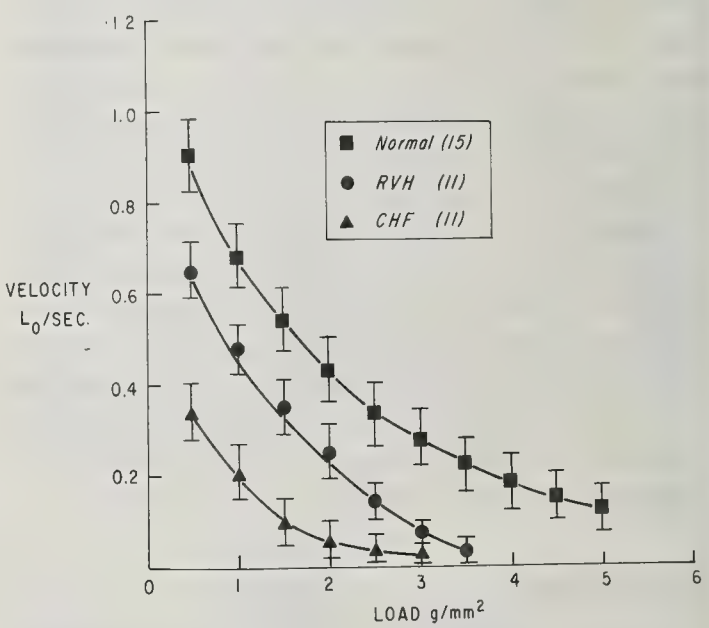


FIGURE 6.—The force-velocity relations of the three groups of cat papillary muscles. Average values with ± SEM are given for each point. Velocity has been corrected to muscle lengths per second (L_o/sec). Numbers in parentheses = number of animals. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* (21:341, 1967.)

is reduced, its duration remains unchanged, since the duration of the active state has been found to be directly related to the time to peak tension in isolated heart muscle.[19] The finding that the time to peak tension is normal indicates that the depression of force in the failing heart muscle does not result from an abbreviation of the duration of active state, but only from a depression of maximal intensity of the active state. Furthermore, had the time to peak tension been increased in the face of the reduction of the rate of tension development, the maximum force the muscle was capable of developing might have been preserved, at least in part. Since this potential compensatory mechanism was not utilized, the maximum isometric force developed by the muscle from the cats with failure was markedly reduced.

Force-velocity relations were examined in muscles from the three groups of animals: normal, right ventricular hypertrophy, heart failure (Figure 6). The initial muscle length were set by a constant small pre-load, and the effects on the velocity of shortening of progressively increasing a load was then determined. At each load the velocity of shortening was less in muscles from both those cats with hypertrophy and those with failure, and the entire force-velocity curve was shifted downward and to the left. The maximal velocity of shortening (V_{max}) at a load of 0.5 gm./mm. sq. averaged 0.09 ± 0.08 muscle lengths/second in normal muscles and was significantly lower averaging 0.65 ± 0.60 muscle lengths/second in the muscles from cats with hypertrophy. V_{max} was significantly lower in muscles from the cats with failure than in muscles from normal cats or those with hypertrophy; the average value in cats with failure was 0.34 ± 0.06 muscle lengths/second. Thus, in cardiac muscle isolated from the hypertrophied and failing heart, there is a severe depression of the contractile state which is reflected by a downward shift of the force-velocity curve with substantial reduction of the intrinsic speed of contraction (V_{max}). These findings further indicate a depression of the intensity of the active state in the muscle from the failing and hypertrophied heart. Also, it must be emphasized that all of these abnormalities are intrinsic properties of the cardiac muscle of the failing heart and are not dependent on the altered humoral or environmental factors, since they are present in vitro; and therefore, the chemical and physical conditions are controlled in an identical manner for all the groups studied.

Inotropic Interventions

After studying the abnormalities of the contractile state in hypertrophied and failing muscle, it was of interest to determine the reaction of these muscles to various positive inotropic interventions. The effects of paired electrical stimulation, changes in frequency of contraction and the addition of strophanthidin were therefore examined.

Sustained post-extrasystolic potentiation produced by paired electrical stimulation has been shown to be a powerful, positive inotropic intervention. However, the depression of the contractile state of the failing heart muscle could not be completely corrected by sustained paired electrical stimulation (Figure 7). Specif-

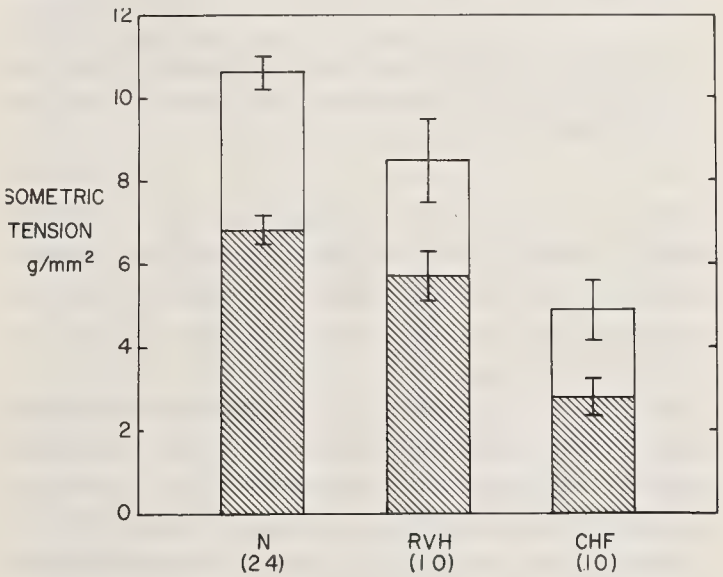


FIGURE 7.—Effects of sustained postextrasystolic potentiation produced by paired electrical stimulation on the maximal isometric force of contraction at L_{max} in the three groups of muscles. The hatched area represents control force with single electrical stimulation; the clear area above represents the augmentation with sustained paired electrical stimulation. Numbers in parentheses = number of animals. Vertical lines with cross bars = ± 1 S.E.M. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* 21:341, 1967.)

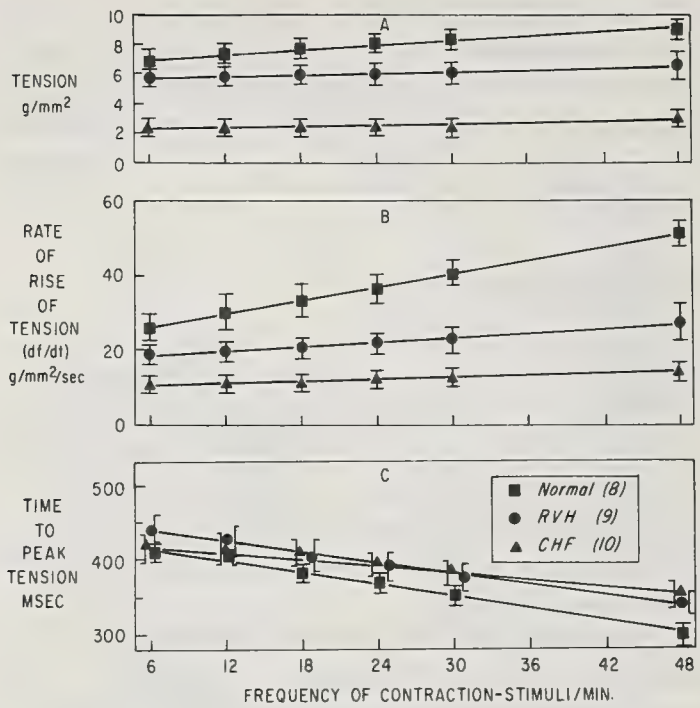


FIGURE 8.—Force-frequency relations in the three groups of muscles. Average maximum isometric tension (top), rate of tension development (middle), and time from stimulation to peak tension (bottom) are shown as functions of the frequency of contraction. The numbers in parentheses = number of animals in each group. Vertical lines with cross bars = ± 1 SEM. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* 21:341, 1967.)

ically, the increments in tension produced by paired electrical stimulation were significantly reduced from the normal value of 3.8 ± 0.3 gms./mm. sq. to 2.2 ± 0.4 gms./mm. sq. in the muscles from cats with failure.

When the frequency of contraction was progressively increased from 6 to 48 stimuli/minute in muscles obtained from normal, hypertrophied and failing cats, there was a progressive elevation in both rate of tension rise and tension (Figure 8) ; whereas, the time from stimulation to peak tension was shortened. However, the increments in the rate of rise of tension in response to changes in frequency were severely depressed in the muscles from the hypertrophied and failing hearts. The increase in the rate of rise per increment in frequency per minute averaged 0.43 gms./mm. sq./second, 0.13 gms./mm. sq./second and 0.07 gms./mm. sq./second in the muscles from normal, hypertrophied and failing hearts respectively. Thus, the positive inotropic effects of increasing frequency of contraction were muted in hypertrophied and failing heart muscle.

The third positive inotropic intervention was the addition of strophanthidin to the muscle contracting isometrically at the apex of the length-tension curve. Strophanthidin, 1.0 micrograms/ml., was added to the bath and the maximal effect, which occurred 20 minutes later, was recorded. Following strophanthidin, the maximal tension achieved was 0.7 gms/mm. sq. a value significantly less than the normal value of 8.4 ± 0.5 gms./mm sq. (Figure 9).

Thus, in addition to the depression of the functional level of the contractile state, muscles obtained from animals with ventricular hypertrophy and heart failure also have depressions of response to positive inotropic procedures. The absolute changes in force and rate of force development produced by paired electrical stimulation increased frequency of contraction and strophanthidin were less in muscles from hypertrophied and failing hearts than in muscles from normal hearts. Furthermore, although the

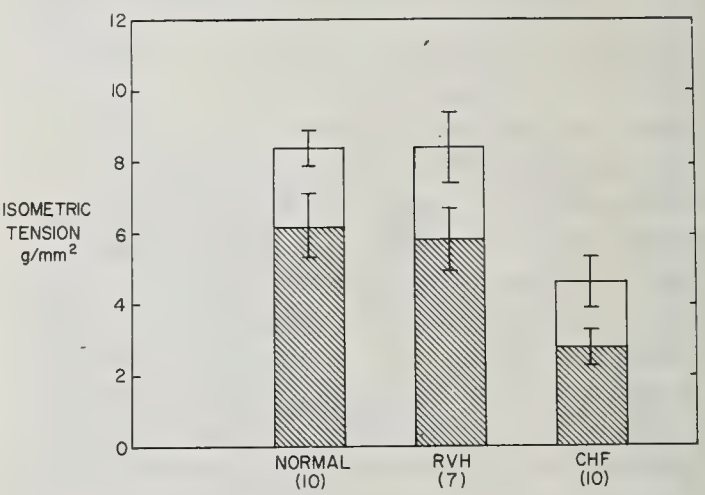


FIGURE 9.—Effects of the addition of 1.0 μg/ml strophanthidin on maximum isometric force of contraction at L_{max} in the three groups of muscles. The hatched area of each bar represents the average control force, the clear area represents the average increment following strophanthidin; the upper limit of each bar indicates the average maximum isometric tension achieved following strophanthidin. Vertical lines with cross bars = ±1SEM; numbers in parentheses = number of animals in each group. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* (21:341, 1967.)

absolute levels of force and rate of force development in the muscles from failing hearts were augmented by these procedures, they did not reach even the basal level observed in normal muscles. Thus, the contractile state is reduced even in the presence of potentiation and the intrinsic defect in heart failure can be only partially corrected by these positive inotropic procedures.

Biochemical Abnormalities

Considerable attention has been devoted to a search for a specific biochemical abnormality responsible for the altered performance of failing myocardium. It is useful to divide the possible abnormalities of myocardial energy supply and utilization into the following areas: substrate availability, energy production, energy storage, energy utilization and conversion, and excitation-contraction coupling.

Substrate Availability. It is known that over a 24-hour period, fatty acids are normally the principal substrate of the energy metabolism of the heart. Recent investigation in isolated preparations of myocardial homogenates have demonstrated a lesion that appears to prevent the full utilization of fatty acids by the failing myocardium.[20] In the heart, metabolism of fatty acids requires a carnitine-dependent system that allows the entrance of free fatty acid into the mitochrondria for beta oxidation. In preparations from the failing heart of experimental animals, this carnitine-dependent system is depressed to one half of normal. It has not been proved, however, that this observed defect is causally related to the abnormalities of performance in the failing heart. Other studies have concluded that fatty acid metabolism is normal in the failing heart.[21] Further work is needed to evaluate these conflicting views.

Energy Utilization. In cardiac mitochrondia, the energy from substrate oxidation is converted into the terminal bond energy of creating phosphate (CP) and adenosine triphosphate (ATP). This process, oxidative phosphorylation, has been studied extensively in animals and man, and more recent data indicate that it is not impaired in heart failure.[22]

Energy Storage. The terminal bond energy resulting from oxidative phosphorylation is stored in the myocardium in the form of CP which serves as a reservoir to maintain ATP, the more immediate source of chemical energy for the heart muscle. The energy stores of CP are depressed in chronically failing hearts of experimental animals. Since the depression of high energy phosphate stores can be experimentally disassociated from the depression of contractibility, however, depression of energy stores is not thought to be a primary cause of the weakness of the failing heart muscle.[23]

Energy Utilization and Conversion. To date there have been no conclusive demonstrations of the alteration in the physical properties of the contractile proteins from failing heart muscle. In addition, the experimental failing heart muscle is not inefficient in the conversion of chemical energy to mechanical work.[24] On the other hand, the activity of myofibrillar ATPase, which splits the terminal phosphate bond off ATP and thereby liberates the energy from the contractile process, is severely depressed in the failing heart and may reduce the rate of energy release.[25]

Excitation-Contraction Coupling. The final biochemical area to be considered is excitation-contraction coupling which occurs within the sarcoplasmic reticulum surrounding each myofibril. Since the release of calcium ions from the sarcoplasmic reticulum into the vicinity of the contractile protein is of fundamental importance for cardiac muscle contraction, a metabolic defect in this area has been proposed as a basic mechanism in the failing myocardium. Thus, it is possible that a fundamental subcellular abnormality responsible for myocardial failure is related to an inadequate release of calcium from the sarcoplasmic reticulum, which could result in improper excitation-contraction coupling and weak contraction. Data to support or reject this hypothesis are not yet available.

From these observations, it can be seen that many areas in biochemistry of the experimental failing heart have been evaluated, a number of possible lesions have been excluded, and although considerable understanding has been gained, no single basic mechanism underlying the depressed performance of the failing heart has been elucidated with certainty. It appears

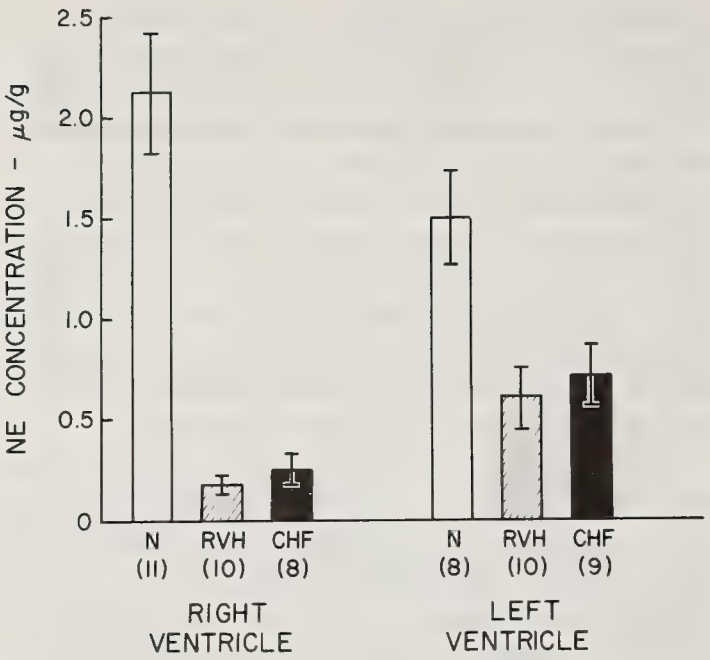


FIGURE 10.—The average norepinephrine concentration of the right and left ventricles of cats in the normal, RVH and CHF groups. Vertical lines with cross bars = ± 1 SEM. Numbers in parentheses = number of animals in each group. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* 21:341, 1967.)

likely that there are multiple abnormalities in the metabolic processes of the experimentally failing heart. At present it can be concluded that at least the following two abnormalities are present: (1) a depression of the failing heart's ability to utilize fatty acid, and (2) a depression of myofibrillar ATPase. In addition, many workers have postulated that there may be a decrease in the release of calcium by the sarcoplasmic reticulum.

Myocardial Norepinephrine Concentration and Response

Marked depression of the cardiac norepineprine concentrations was observed in both the right and left ventricles of the right ventricular hypertrophy and congestive heart failure groups. The extent of depression was comparable in both of these experimental groups (Figure 10). The inotropic effect of L-norepinephrine was studied in the muscles from eight normal cats and six cats with pulmonary artery constriction. Four of these were in the right ventricular hypertrophy group and the other two in the congestive heart failure group, but because of the small size of these two groups and the fact that all six hearts showed severe depletion of right ventricular norepinephrine to a concentration of 0.39 micrograms/gm. or less (norepinephrine concentration in these right ventricles averaged 0.18 ± 0.12 micrograms/gm.), they were analyzed as a single group and compared to the normal. All muscles responded to norepinephrine with an increase in tension and the increments were greater in muscles from the cats in the right ventricular hypertrophy and congestive heart failure groups (Figure 11). For example, at the

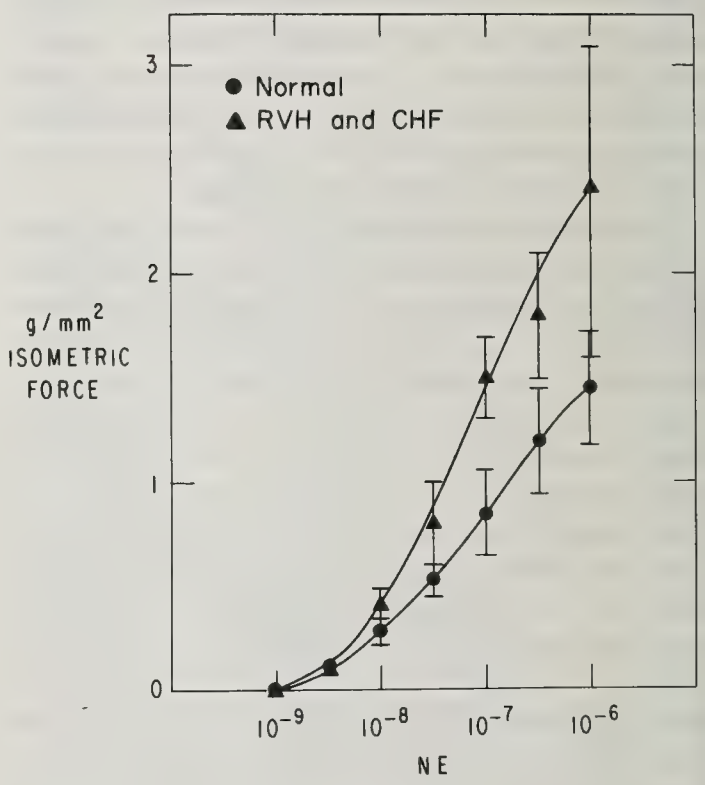


FIGURE 11.—Effects of exogenous norepinephrine on isometric tension of papillary muscles of cats in the RVH and CHF groups. l-norepinephrine was added to the muscle bath at 5-min. intervals in increasing concentrations starting at 10^{-10}M, while isometric tension achieved at each concentration was recorded at L_{max}. The increment in isometric tension is shown on the ordinate and the concentration of added norepinephrine (NE) on the abscissa. Solid circles = muscles from normal hearts; solid triangles = muscles from the norepinephrine-depleted hearts of animals in the RVH and CHF groups. Vertical lines with cross bars = ± 1 SEM. (Reproduced with permission from the American Heart Association, Inc. from *Circulation Res.* (21:341, 1967.)

concentration of 10 molar of L-norepinephrine, the increment in isometric force averaged 1.47 ± 0.23 gms./mm. sq. in muscles from cats of the right ventricular hypertrophy and congestive heart failure groups, a value significantly greater than that observed in the normal muscles, 0.84 ± 0.2 gms./mm. sq. Thus, the dose response curve was shifted upward, signifying an increased response to norepinephrine; the catecholamine depleted cardiac muscle from the hypertrophied and failing heart is supersensitive to the positive inotropic effects of exogenous norepinephrine.

Contractility in the Intact Ventricle

Since the previously described investigations of contractile performance were carried out on isolated muscles only, under conditions quite different from those existing in the intact animal, the contractile mechanics of the intact right ventricle of cats in which heart failure, produced as described before, were also studied. The protocol followed in these experiments was as follows: The hemodynamic status of each cat was determined by right heart catheterization, the chest was then opened and an isovolumic contraction was then produced by total occlusion of the pulmonary artery in the preceding diastole. Simultaneous measurement of the right ventricular pressure and the rate of pressure rise (dP/dt) allowed determination of the maximum velocity of shortening (V_{max}) and the maximal isometric tension per muscle unit in the intact ventricle.[26-29] This allowed a description of the mechanical performance of the heart muscle of the intact ventricle within a framework of the velocity of shortening, muscle length and tension and provided a quantitative description of the ventricular performance within a construct of muscle mechanics as it has been previously described in isolated papillary muscle. Furthermore, to study the two groups of hearts at comparable end-diastolic volumes, the animals with failing hearts were bled to decrease the end-diastolic volume and the normal animals were infused to increase end-diastolic volume; the isovolumic contractions were repeated at various points along the Frank-Starling curve of each ventricle. In this manner, a segment of the length-tension curve could be described for each ventricle, *in vivo*. After the determination of ventricular function, the papillary muscles of these ventricles were removed through the tricuspid valve for study. The ventricular wall was left intact, and passive pressure volume curves were obtained in each right ventricle.

The maximum velocity of shortening was markedly reduced in the ventricular muscle of the intact, failing heart and the whole force-velocity relationship was shifted downward and to the left in the failing ventricle, a finding exactly like that seen in the isolated papillary muscle. The maximum velocity of shortening (V_{max}) in the intact ventricle, obtained by extrapolation, was depressed 40 percent below the normal value in both hypertrophied and failing hearts. The peak isovolumic ventricular wall tension at spontaneous end-diastolic volumes was only slightly reduced from normal in the animals with hypertrophy and failure; whereas, the right ventricular end-diastolic tension and spontaneous end-diastolic volume was elevated to twice the normal value. The normalized right ventricular end-diastolic volume and circumference from which this spontaneous resting and active tension were recorded were significantly elevated from the normal value in these animals.

When the length-tension curves were obtained by manipulation of end-diastolic volume in the contracting right ventricles of the failing and normal hearts, it could be seen that the intact, failing ventricle, like the failing papillary muscle, is operating along an ascending limb of a generally depressed length active tension curve and not along the descending limb of a normal curve. In fact, when comparisons were made at similar end-diastolic normalized circumferences, active tension was reduced to one half of the normal value in both hypertrophied and failing hearts. By operating to the right along its depressed length-tension curve, the failing ventricle can compensate, at the expense of increased end-diastolic volume, pressure and tension, and can maintain and even exceed the isometric tension usually developed by the normal ventricle at its smaller end-diastolic volume.[30] The cardiac output was often normal

in animals with severe depression of ventricular performance. From these studies in the intact ventricle of normal, hypertrophied and failing hearts, it is concluded that the observations made in the isolated papillary muscle were also true for the intact ventricles of these animals. There was obvious depression of myocardial function both in isolated muscle and intact ventricles of animals with hypertrophy but without overt heart failure in which the cardiac output was normal. Thus, it is apparent that depression of the cardiac output is a late finding in congestive heart failure.

SUMMARY

Pulmonary artery constriction in the cat has been and continues to be an excellent model for investigation of ventricular hypertrophy and congestive heart failure. By this method some aspects of the pathophysiology and the clinical syndrome of congestive heart failure have been elucidated. It has been found that the contractile state of isolated cardiac muscle in both the hypertrophied and failing heart is severely reduced. It is also apparent that there is severe depression of the contractile state of the intact ventricle of experimental animals in which there is hypertrophy but no overt congestive heart failure and in which the cardiac output is normal at rest. It has become increasingly important to determine the contractile state of the myocardium in cardiac disease, since its proper understanding provides the basis for the appreciation of the compensatory mechanisms necessary to maintain cardiac output in the face of severely reduced myocardial contractility.

Also this cat pulmonary constriction heart failure model has provided tissue for investigation of the biochemical abnormalities associated with the failing heart. Disorders of lipid substrate utilization, myofibrillar ATPase, and the sarcoplasmic reticulum-calcium system have been demonstrated or implied, and several potential lesions have been excluded. Both sympathetic nervous system function and cardiac catecholamine metabolism are strikingly altered in ventricular hypertrophy and in congestive heart failure. The depletion of myocardial catecholamine results in the impairment of a potential compensatory mechanism. In this situation, however, circulating catecholamine represents an important support for the failing heart, which is supersensitive to the effects of exogenous norepinephrine, a fact that explains the deleterious effect that may occur when beta-adrenergic receptor blocking agents are administered to patients with depressed cardiac function.

In addition to the changes in the central circulation, there are important changes in the peripheral arterial and venous beds in heart failure that aid in the maintenance of circulatory function. The cardiac compensatory mechanisms that help maintain circulation in the face of the depressed intrinsic state of the myocardium can be considered in terms of the resultant symptoms that they produce. The principal compensations are by myocardial hypertrophy, the Frank-Starling mechanism, and altered sympathetic nervous system activity to the heart, arterial and venous beds. Each compensatory mechanism has a built-in set of limitations that, when exceeded, produce signs and symptoms, such as dyspnea, pulmonary edema and systemic venous congestion. A reduction in resting cardiac output is a relatively late manifestation of the failing heart; when the compensatory mechanisms are no longer sufficient to overcome the intrinsic depression of the contractile state, the cardiac output fails even at rest, and the end stages of congestive heart failure are apparent in weakness, mental confusion, oliguria and hypotension.

REFERENCES

1. Barger, A. C., Richardson, G. S., Roe, B. B. A method for producing chronic cardiac failure in dogs. Proc. Soc. Exp. Biol. & Med. 73:113, 1950.
2. Gertler, M. M. Production of experimental congestive heart failure in the guinea pig. Proc. Soc. Exp. Biol. & Med. 102:396, 1959.
3. Alexander, N., Edmonson, H. A., Druary, D. R. The production of experimental congestive heart failure in rabbits. Circ. Res. 1:491, 1953.
4. Nadeau, R. A., Colebatch, J. J. H. Normal respiratory and circulatory values in the cat. Appl. Physiol. 20:863, 1965.
5. Sonnenblick, E. H. Implications of muscle mechanics in the heart. Fed. Proc. 21:975, 1962.

6. Spann, J. F., Jr., Buccino, R. A., Sonnenblick, E. H., and Braunwald, E. Contractile state of cardiac muscle obtained from cats with experimentally produced ventricular hypertrophy and heart failure. Circ. Res. 21:341–354, 1967.
7. Pool, P. E., and Sonnenblick, E. H. Mechanochemistry of cardiac muscle: I. The isometric contraction J. Gen. Physiol. 50:951, 1967.
8. Pool, P. E., Chandler, B. M., Seagren, S. C., and Sonnenblick, E. H. Mechanochemistry of cardiac muscle: II. The isotonic contraction. Cir. Res. 22:465, 1968.
9. Hume, B. J. The influence of growth hormone on the protein composition of rat muscle. Biochem. J. 52:XI, 1952.
10. Harkness, M. L. R., and Harknes, R. D. The distribution of the growth of collagen in the uterus of the pregnant rat. J. Physiol., London 132:492–495, 1956.
11. Blumgart, H., Gilligan, D., and Schlesinger, M. The degree of myocardial fibrosis in normal and pathological hearts as estimated chemically by the collagen content. Trans. Assoc. Am. Physicians 55:313, 1940.
12. Montfort, I., and Perez-Tamayo, R. The muscle-collagen ratio in normal and hypertrophic human hearts. Lab. Invest. 11:463–470, 1962.
13. Oken, D. E., Boucek, R. J. Quantitation of collagen in human myocardium. Circ. Res. 5:357–361, 1957.
14. Van Slyke, D. D., Neill, J. M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. Biol. Chem. 61:523, 1924.
15. Spann, J. F., Jr., Chidsey, C. A., Pool, P. E. and Braunwald, E. Mechanism of norepinephrine depletion in experimental heart failure produced by aortic constriction in the guinea pig. Circ. Res. 12:312, 1965.
16. Nair, K. C., Cutilletta, A. F., Zak, R., and Rabinowiz, M. RNA polymerase activities in hypertrophied and normal rat heart. Clin. Res. 15:217, 1967.
17. Sonnenblick, E. H. Force-velocity relations in mammalian heart muscle. Am. J. Physiol. 202:931, 1962.
18. Parmley, W. W., Spann, J. F., Jr., Taylor, R. R., and Sonnenblick, E. H. The series elasticity of cardiac muscle in hyperthyroidism, ventricular hypertrophy, and heart failure. Proc. Soc. Exp. Biol. & Med. 127:606–609, 1968.
19. Sonnenblick, E. H. Active state in heart muscle: Its onset and modification by inotropic interventions. J. Gen. Physiol 50:661, 1967.
20. Wittels, B., Spann, J. F., Jr. Defective lipid metabolism in the failing heart. J. Clin. Invest. 47:1787–1794, 1968.
21. Blain, J. M., Schafer, H., Siegel, A. L., and Bing, R. J. Studies on myocardial metabolism: VI myocardial metabolism in congestive failure. Amer. J. Med. 20:820–833, 1956.
22. Sobel, B. E., Spann, J. F., Jr., Pool, P. E., Sonnenblick, E. H. and Braunwald, E. Normal oxidative phosphorylation in mitochondria from the failing heart. Circ. Res. 21:355–363, 1967.
23. Pool, P. E., Spann, J. F., Jr., Buccino, R., Sonnenblick, E. H., and Braunwald, E. Myocardial high energy phosphate stores in cardiac hypertrophy and heart failure. Cir. Res. 21:365–373, 1967.
24. Pool, P. E., Chandler, B. M., Spann, J. F., Jr. Mechanochemistry of cardiac muscle IV. Utilization of high energy phosphates in experimental heart failure in cats. Circ. Res. 24:313–320, 1969.
25. Chandler, B. M., Sonnenblick, E. H., Spann, J. F., Jr., and Pool, P. E. Association of depressed myofibrillar adenosine triphosphate and reduced contractility in experimental heart failure. Circ. Res. 21:717–725, 1967.
26. Fry, D. L., Griggs, D. M., Jr., and Greenfield, J. C., Jr. Myocardial mechanics: Tension-velocity-length relationships of heart muscle. Circ. Res. 14:73, 1964.
27. Levine, H. J. and Britman, M. A. Force-velocity relations in the intact dog heart. J. Clin. Invest. 43:1383, 1964.
28. Ross, J., Jr., Covell, J. W., Sonnenblick, E. H., and Braunwald, E. Contractile state of the heart characterized by force-velocity relations in variably afterloaded in isovolumic beats. Circ. Res. 18:149, 1966.
29. Taylor, R. R., Ross, J., Jr., Covell, J. W. and Sonnenblick, E. H. Quantitative analysis of left ventricular myocardial function in the intact, sedated dog. Circ. Res. 21:99, 1967.
30. Spann, J. F., Jr., Covell, J. W., Eckberg, D. L., Sonnenblick, E. H., Ross, J., Jr., and Braunwald, E. Myocardial contractile state in hypertrophy and congestive failure: Studies in the intact heart and the isolated muscle. Circ. 36 (Suppl. II) :241, 1967.

DISCUSSION

Chairman La Farge: Thank you very much Dr. Spann for the usual elegant presentation of ventricular dynamics in the normal and abnormal state.

W. B. Wead, University of Louisville, Ky.: I was interested in your fine presentation, especially the diastolic compliance part of your tension curve. Did you try paired stimulation and if so, what were the results in comparison between the hypertrophied and the failed heart?

Dr. Spann: Yes, we did use sustained paired

stimulation in each of the papillary muscles that we studied and they were potentiated in a percentage similar to the normal muscle. For example, let us assume that the normal tension was 1. You paired stimulation and it went up to 2. Of course, those are not actual figures. In the failure animal, it was 0.1 and would go up to 0.2. So the percentage rise was identical. However, none of the positive inotropic interventions such as paired stimulation, digitalis, epinephrine, and calcium added together, would bring the failing papillary performance up to the nonpotentiated baseline level of the normal papillary muscle under any circumstances. We could raise it, but not up to the normal level. Regarding the first part of your question, I am convinced, incidentally, that this is not the way to measure compliance. We haven't said much about this because I don't know what to make of it. They look stiffer but they don't measure.

CHAIRMAN: In your opinion, what is the mechanism of the problem in question? I ask because I am wondering about the left ventricular changes observed in the presence of the right ventricular. Is this a dominant left coronary system in the cat and if so, could there be some involvement of the adjacent free wall of the left ventricle to the septum which could be limiting the left ventricle by the massive hypertrophy which you obtained?

DR. SPANN: That is a possibility. I don't think so from the electron microscopic studies. The mitochondrial crista which, I believe, are fairly sensitive indicators of oxygen deprivation, are completely intact. There is no scar, no evidence of myocardium degradation. We looked at hundreds of sections of these and obtained no information whatsoever different from the normal. It was very discouraging.

W. B. HOOD, Boston City Hospital: Most of the isolated muscle data were normal on a cross-sectional area. When you followed the anatomy of the situation and compared muscle with muscle, was there a stage of failure where the hypertrophied muscle actually compensated for its overload in the isolated studies, that is if you considered the whole muscle function.

DR. SPANN: We did not look, as Meerson did, for a hyperstage before depression. We did not cross section it by time in order to find that out. We did not have enough points to answer that thoroughly. Considering the tension developed by the right ventricle, at the working end-diastolic pressure before we bled them or infused them, then the tensions were comparable per unit across the sectional area. In other words, they were working at the same wall tension before we intervened in the hypertrophy and failure group as compared to normal. As for the papillary muscles, they were not comparable. Without correcting for the cross sectional area, the value would still be at least half normal, even when taking just the papillary muscle into consideration. This, however, poses problems since you can select a papillary muscle the size of your finger or the size of your thumb, depending on which one you pull out of the right ventricle. Therefore you could not be sure how big it was before it hypertrophied. It might have been a tiny papillary muscle and I would not know which one of the normals to compare it to.

E. W. HAWTHORNE, Howard University College of Medicine, Washington, D.C.: Dr. Spann, how soon after your banding did the right ventricular end-diastolic volume increase? I raise that question with reference to an earlier comment indicating that this was presumably a pure pressure overload experiment.

DR. SPANN: The fact is, the moment you put the clip on the pulmonary artery the ventricular volume approximately doubled and it seemed to maintain a similar volume throughout the rest of the experiment. I'm guessing now when stating that it doubled. The ventricle does dilate quite strikingly and it seems to stay that way. But we did not examine them eight to twelve hours later. Perhaps there is a stage when they compensated, become normal in volume, and then increased later. I can't answer that. Some in the hypertrophy group were not dilated at all when open chest measurements were taken after sacrificing the animal.

CHAIRMAN: Dr. Hawthorne's precise observations leave me almost speechless. What I was trying to say, Dr. Hawthorne, was that this preparation was generated by a pressure overload in contrast to the original Barger dog where tricuspid insufficiency as a volume load was used from the very beginning in combina-

tion. You're quite right. The ventricle dilates instantaneously, or virtually so.

DR. HAWTHORNE: The first thing that happens is you have a volume which can't get out. The pressure problem comes after the fact. To my knowledge the main problem is how do we get a preparation where pure pressure overload exists; so far none exists.

DR. SPANN: What do you call a volume overload? Diastolic volume is certainly up, but the stroke volume in these animals is normal, unlike a shunt.

DR. HAWTHORNE: Well, a pure volume overload would imply that you stressed the resting sarcomere length out beyond 2.2 microns if you are thinking in terms of contractile elements.

H. L. BROOKS, University of Chicago: I became interested in the same problem, particularly in the acute right ventricular stress overload, as in a pulmonary embolus, and I wondered if you had looked at left ventricular dynamics as an explanation for the increased hydroxy proline levels which you noticed in the left ventricular free wall? If so, is there some mechanism to explain these increased levels' involvement of the left ventricle?

DR. SPANN: No, we have no data at all in terms of instantaneous length and shortening and V-max on the left ventricle. The only facts we have on the left ventricle are the overall hemodynamics, i.e., the fact that the cardiac output was normal, that by implication the constriction is over on the pulmonary artery and that the left ventricle does not hypertrophy. I also wish to point out that the norepinephrine changes were striking in the left ventricle. They were even more pronounced than the hydroxyproline changes on a percentage basis. This has, of course, been observed by others. Dr. Charles Chidsey first observed it in the dog when using the Barger preparation.

EXPERIMENTAL MYOCARDIAL INFARCTION BY CLOSED CHEST TECHNIQUE

E. L. Stanley, P. Kezdi and R. K. Kordenat*

This paper describes our experience in development and evaluation of a myocardial infarction model designed to closely resemble the human condition. Proximal coronary artery occlusion occurs in the awake, intact dog by gradual thrombosis occurring from two hours to two days, depending on the type of metallic material used. Copper alloys clotted in one to two hours while magnesium alloys clotted over a period of 48 hours.

The sequential electrophysiologic and hemodynamic changes produced by the myocardial infarction were evaluated from ECG's, aortic blood pressures, cardiac output by dye dilution technique, and enzyme determination. In 43 dogs the helical coil wire was placed in either the left anterior descending branch (24 animals) or the left circumflex artery branch (19 animals). Serial ECG's showed tachycardia followed by A-V dissociation and idioventricular rhythm and PVC in six to eight hours. S-T segment depression progressed to S-T segment elevation and Q-waves were noted by 24 hours. The A-V dissociation persisted for 48 hours and then reverted back to sinus rhythm. Sixty-three % of the animals survived greater than 48 hours with most of the early deaths due to the untreated arrhythmias. The cardiac output decreased 35% and the blood pressure 30% with a gradual return towards preinfarction levels. SGOT's were elevated (300 units). Post mortem examination showed complete occlusion at the helical wire site and a myocardial infarction that closely resembled the human condition. The average infarction weight was 22 grams (average heart weight of 150 grams). This model closely resembled the human condition; coronary artery occlusion occurred by thrombosis, a small myocardial infarction with arrhythmias developed and a 70% survival rate with gradual return of the hemodynamic values toward normal was noted.

INTRODUCTION**

Numerous models of experimental myocardial infarction have been developed in the past.[1-3] No one of these models however simulated closely the clinical myocardial infarction. An ideal model should include: (1) single or multiple segmental occlusions of the coronary artery by a gradual occluding process without thoracotomy; (2) a predictible time of myocardial infarction in the awake intact animal and; (3) electrophysiologic, hemodynamic, and prognostic results similar to the experience in the human condition. The ideal model would also permit sequential studies in the intact animal of the events prior to, during, and with recovery from myocardial infarction, and the efficacy of measures for its prevention and treatment.

The present paper describes our experience in development and evaluation of a myocardial infarction model that was designed to closely resemble the human condition. Proximal coronary occlusion occurs in the awake, intact animal by gradual thrombosis following insertion of a helical coil wire into a coronary artery.[4] The technique of the helical coil wire insertion, sequential electrophysiologic and hemodynamic measurements, and general applications of this model are described in the paper.

METHODS AND MATERIALS

In this study, mongrel dogs of both sexes weighing from 15–25 kg were used. A total of 71 dogs were studied. The animals were sedated with morphine sulfate and, under sterile conditions, the left carotid artery and jugular vein were exposed. A special purpose double catheter system that permits selective catheterization of the left circumflex and anterior descending branches of the left coronary artery was passed under x-ray visualization to the root of the ascending aorta (Figure 1). This special purpose catheter, constructed by us, consists of a catheter within a catheter. The outer preformed catheter rests against the ascending aortic wall and points the inner catheter into the left coronary artery. The larger catheter (Formacath

* Cox Heart Institute, 3525 Southern Boulevard Dayton, Ohio

** This work was supported by NIH Contract PH-43-68-688 and Grant HE09885.

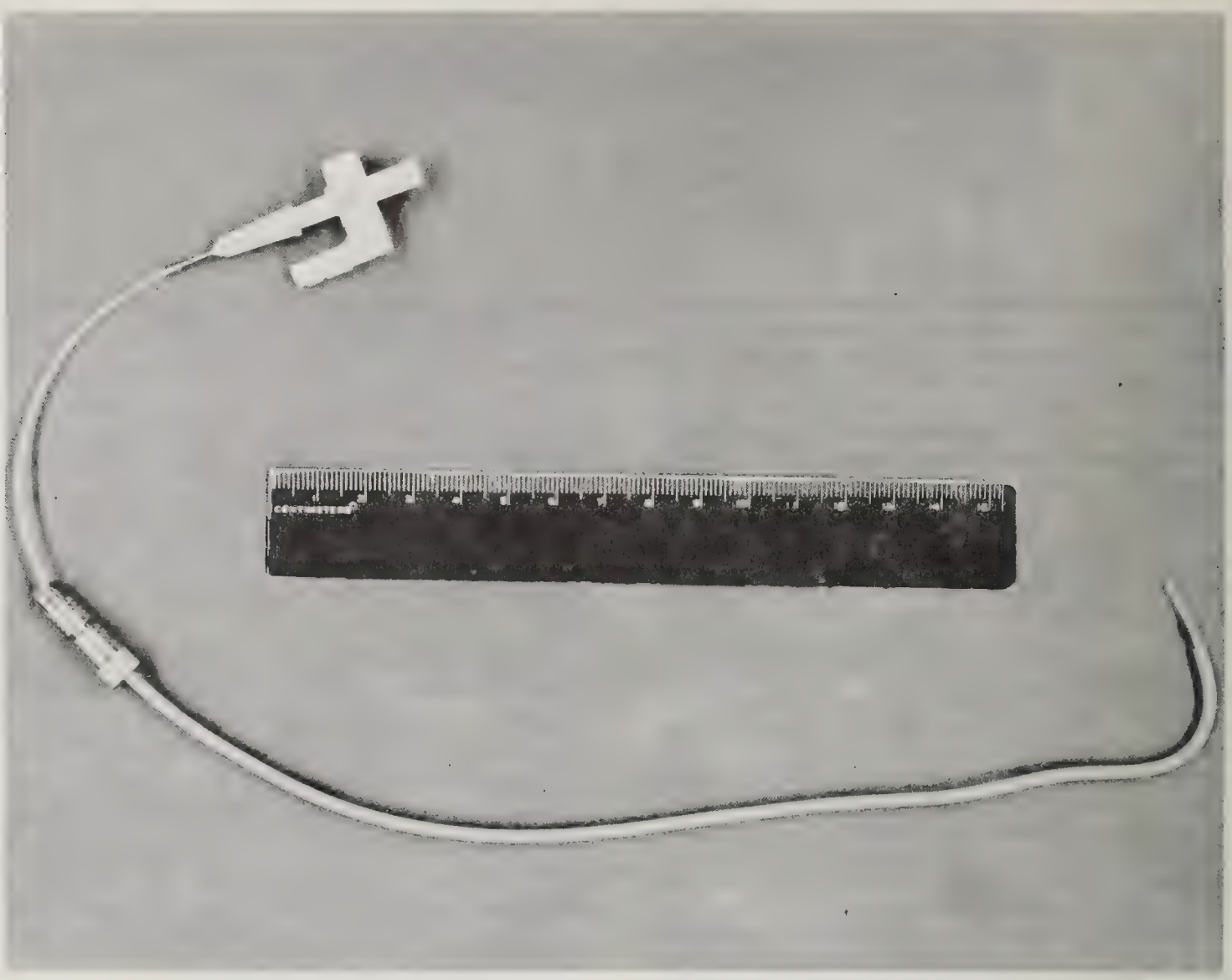


FIGURE 1.—The double catheter system and the helical coil wire that fits snugly over the inner catheter. Release of the wire is achieved by forward thrust of the outer catheter against the coil.

#7752) was previously shaped with 45° curves at 2.5 cm and 0.9 cm from the tip. The first curve braces against the aortic wall and stabilizes the tip. The smaller catheter (P.E. 90) is then advanced while slowly rotating the outer catheter until the left coronary ostia is identified. The inner catheter is then further advanced down the main left coronary branch and its position verified by injection of contrast media. The helical coil wire placed over the inner catheter is released by forward pressure of the outer catheter. The helical coil consists of a magnesium alloy wire 0.02″ diameter, which is formed into a spring-type coil by winding around a 18–20 gauge needle to form a coil approximately one-half centimeter long. At least four complete turns of the coil within the 0.5 cm length are necessary for the intravascular clotting process to develop. The coil floats freely down the selected coronary artery branch and lodges into position but permits blood flow through its lumen. Gradual occlusion occurs by clotting over the next 12 to 36 hours. The outer preformed catheter can be left in the aorta for recording of blood pressure, withdrawal of samples, and recatheterization of the coronary artery.

Serial ECG's, aortic mean blood pressure, and cardiac output by the dye dilution technique (Cardiogreen, Gilford Model No. 103 IR densi-

tometer) were recorded and blood samples drawn for SGOT determination. Cardiac output was measured in duplicate and the average of the two measurements was used. All animals were evaluated up until the time of death or sacrificed by the fourteenth day.

Postmortem examinations were performed in all animals with measurement of the size and the weight of the myocardial infarction. The helical coil obstructed site was dissected out and histologic sections of the thrombosis obtained. The time of infarction was determined by the ECG changes, elevation of the SGOT, and the appearance of the myocardium at the time of postmortem examination.

The animals studied were divided into three groups. In the first group the consequences of the gradual occlusive process at the helical coil site were evaluated without intervention other than the treatment of serious arrythmias. Coronary occlusion with myocardial infarction occurred 16–24 hours after helical coil insertion in this group of animals. The second group of animals was pretreated with heparin, 4,000 units intravenous in one dose, to evaluate prolongation of occlusion time. This anticoagulation technique with heparin prolonged the average time of occlusion and myocardial infarction to approximately 48 hours. In the third group of animals a shortening of the occlusive time was evaluated by injection of a 0.1% solution of palmitic acid into the coronary artery branch at the site of the helical wire. Immediate occlusion by clotting at the site of the helical wire occurred in one to two minutes.

RESULTS

Clinical Course, Electrocardiographic, and Hemodynamic Changes

In 43 dogs the helical coil wire was placed in either the left anterior descending (24) or the left circumflex artery branch (19). In the left anterior descending branch the wire was distal to the septal but proximal to the first major epicardial branch. Occlusion at the wire site with distal infarction occurred within the first 24 hours in 21 of the 24 animals. One animal died due to arrhythmia one hour after wire placement, and on two occasions the wire was properly positioned, but no clot occurred. Those animals who survived longer than 24 hours lived an average of 11 days with an average infarction weight of 20 gms (Table I).

TABLE I.—*Myocardial Infarction by Helical Wire Placement in 45 Dogs*

	Left Circumflex	Anterior Descending	Total	% of 46 Attempts
Successful	19	24	43	94
Survival > 48 hours	9	18	27	63
Infarction	12	21	33	77
Acute arrhythmia 1 hr.	2	1	3	7
Failure to clot	5	2	7	16
Average survival	8 days	11 days		
Average infarct wt.	22 gm.	20 gm.		
Average heart wt.	154 gm.	152 gm.		

Results of selective placement of helical wires in the left coronary artery using the double catheter technique.

In the 19 animals with wire placement in the left circumflex artery the helical wire was in the proximal 2 cm in 17 of the 19 animals. In two animals the wire descended to the marginal branch and both animals died within one-half hour due to arrhythmia. In five animals the helical wire failed to clot due to the small (less than three coil) size. The average survival time of the animals with left circumflex artery occlusion was eight days with an average infarction weight of 22 gms. Serial ECG's of all animals following wire placement showed a similar pattern of evolution the first 24 hours (Figure 2). There was an initial tachycardia which in a period of six to eight hours increased up to rates of 160 with AV dissociation and idioventricular tachycardia. This AV dissociation and idioventricular tachycardia was not significantly affected by antiarrhythmic agents such as pronestyl or lidocaine whereas the appearance of premature ventricular beats was controlled by these drugs. The initial ST depression progressed to ST elevation and Q waves developed in the appropriate leads of the ECG. The AV dissociation and idioventricular tachycardia persisted for 24–48 hours and in those animals that survived longer than three days reverted back to a normal sinus rhythm. The ST–T wave demonstrated a normal evolutionary pattern with return to the base line within four to ten days.

Serial hemodynamic values were compared in

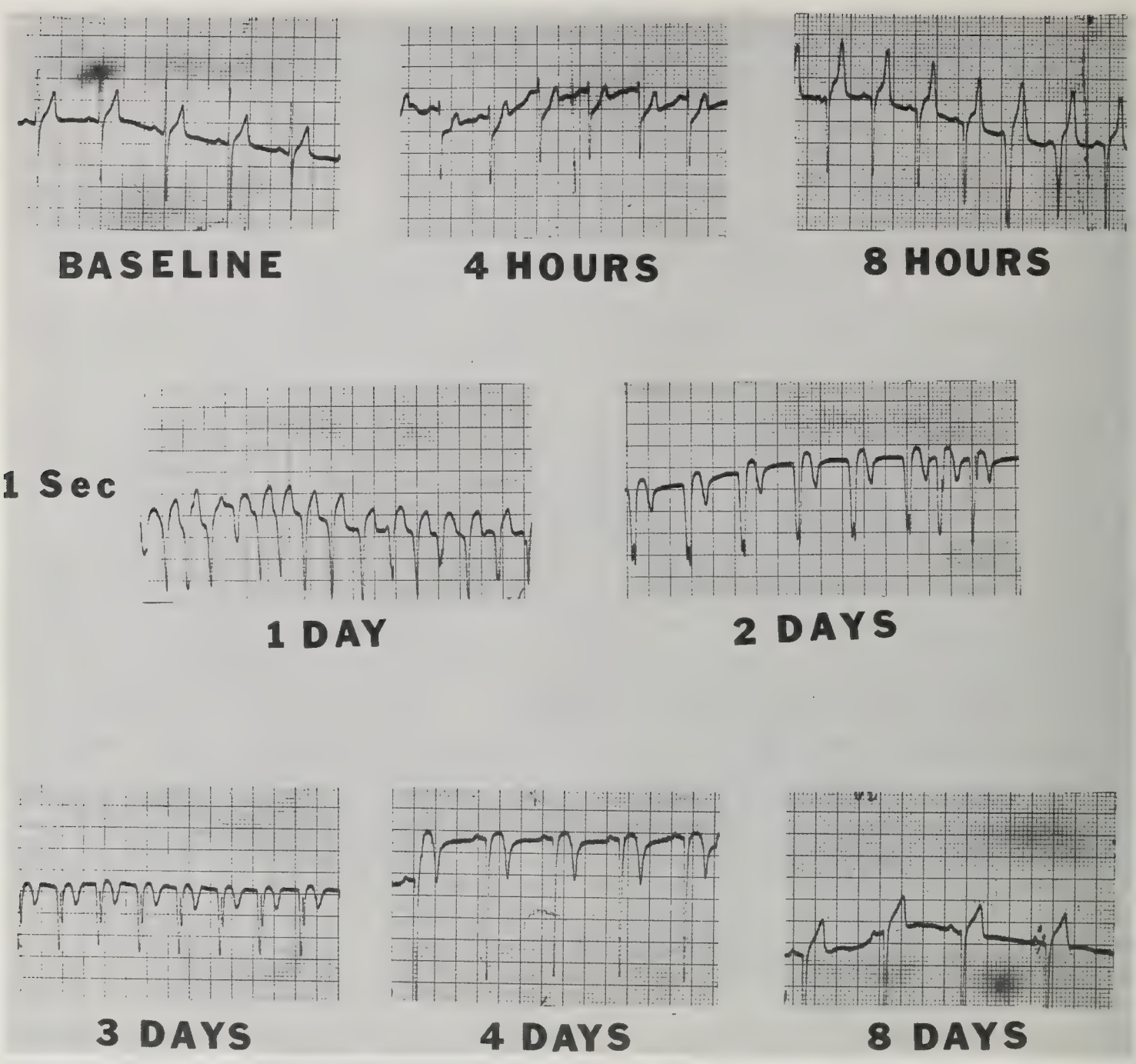


FIGURE 2.—Serial ECG's which demonstrate the initial tachycardia, idioventricular tachycardia, and acute ischemia changes. After 48 hours the rhythm is sinus and the Q waves of acute myocardial infarction are present.

the dogs surviving greater than 48 hours (Table II). A statistically significant decrease occurred in several parameters at both 24 and 48 hours after helical wire implant. The cardiac output and stroke volume were maximally decreased at 24 hours and in survivors gradually improved over the next several days but never returned to the control values. The heart rate was maximal at 24 hours—usually idioventricular tachycardia—and both rate and rhythm reverted to normal by the third day. While the hemodynamic changes and infarction weight were similar in both left circumflex and anterior descending artery occlusions the SGOT elevation was not as high in the former. However the wide range of enzyme values was such that the difference was not statistically significant.

TABLE II.—*Hemodynamic Results Following Experimental Myocardial Infarction Produced by Helical Coil Insertion*

Anterior Descending (10 Dogs)					
	Average Cardiac Output Lit/min.	Heart Rate	Stroke Volume	MBP	SGOT
Control	4.2	115 B/min.	36 cc	127 mm Hg	—
8 hour	2.8	155*	18 cc*	115 mm Hg	—
24 hour	2.2*	195*	12 cc*	81 mm Hg	452
48 hour	2.3*	177*	13 cc*	95 mm Hg	330
Left Circumflex (11 Dogs)					
	Cardiac Output	Heart Rate	Stroke Volume	MBP	SCOT
Control	3.2	96	33	128 mm Hg	—
8 hour	2.4	170	14 cc*	90 mm Hg	—
24 hour	2.1	168*	13 cc*	91 mm Hg	152
48 hour	2.8	158*	19 cc*	98 mm Hg	94

* $p < 0.02$

The hemodynamic values recorded in 21 dogs that survived at least 48 hours following the acute mycardial infarction produced by occlusion of the helical coil.

Rapid Luminal Occlusion

In eleven animals the effects of rapid occlusion was evaluated by the infusion of a 0.1% solution of palmitic acid into the coronary artery shortly after release of the helical coil wire (Table III). One hundred mgms of the Crystaline fatty acid* was dissolved in 100 cc of normal saline heated to 50° centigrade. Two cc of the mixture were slowly injected (one min.) through the inner catheter just proximal to the helical coil while the ECG was continuously monitored. Small amounts of radio contrast media were used to determine the time of complete occlusion.

In nine animals 2–4 cc of the palmitic acid, when injected proximal to the helical wire, produced immediate occlusion at the wire site. Seven of the nine animals died immediately with acute dilatation of the heart in either ventricular fibrillation or electrical standstill. Two animals survived the acute occlusive procedure but died 30 minutes and four hours later, respectively. At post mortem examination the helical wire was completely occluded while the artery distal to the wire site was patent in all animals.

In two animals the fatty acid solution was injected without a helical coil wire being present. After 20 cc total injection was made during a 10 minute period, the ECG showed typical ST-T wave changes of acute ischemia progressing to arrhythmia and death. No occlusion of the major branches of the arteries was seen at postmortem examination.

* Matheson, Coleman, and Bell—Norwood, Ohio

TABLE III.—*Rapid Occlusion of a Helical Wire with 0.1% Palmitic Acid*

Palmitic Acid 0.1%	No. Dogs	Anterior Descending	Left Circumflex
Total	11	6	5
With wire	9	5	4
Without wire	2	1	1
Thrombosis	9	5	4
Time of Death			
Immediate	9	4	5
Later	2	4 hr.	30 min.
ECG Changes			
Ventricular Fibrillation	6	3	3
AV Diassociation	1	1	
Asystole	4	2	2

Injection of 0.1% palmitic acid at the helical wire resulted in immediate occlusion and sudden death. Listed are the time of death and the terminal arrhythmia event.

Prolongation of Luminal Occlusion

In 17 dogs the effect of preheparinization upon the time of luminal occlusion at the wire site was evaluated. The standard technique of cut down, double catheter placement, and wire releases were followed; the only exception being the injection of 4,000 units of sodium heparin I.V. just prior to the release of the helical coil. The animals were evaluated by daily ECG's, SGOT levels, and visualization of the left coronary artery by injection of dye into the branch containing the helical coil.

In ten animals the wire was placed in the anterior descending branch and in seven animals into the circumflex branch (Table IV). The serial selective visualization of the coronary branch showed gradual narrowing of the arterial lumen at the site of the coil progressing to complete occlusion. In eight animals the blockade was complete within the first 24 hours after wire insertion. In seven animals the time

TABLE IV.—*Heparin Induced Prolongation of Helical Wire Occlusion Time of Occlusion (Day after Wire Insertion)*

	Number of Dogs						
Day of Occlusion	1st	2nd	3rd	4th	5th	No Occlusion	Total
Anterior Descending	5	1	1	2	0	1	10
Left Circumflex	3	1	1	0	1	1	7
Total	8	2	2	2	1	2	17
% of Total	47%	12%	12%	12%		5%	—

Delay of the onset of the occlusion at the helical coil site by 4000 units of heparin injected at the time of wire implant. No heparin given following the initial dosage.

of blockade and infarction was delayed up to the fifth post-insertion day, while in two animals no occlusion developed and the arterial lumen was patent up to ten days.

In one of the animals which failed to demonstrate clot formation at the wire site the long chain free fatty acid (palmitic acid) was injected. After 4–6 cc of 0.1% palmitic acid solution there was complete occlusion at the wire site, the ECG demonstrated hyperacute ischemic changes, and the animal promptly fibrillated (Figure 3). Resuscitation efforts were to no avail. At postmortem examination the clot was easily dissected from the helical coil wire but remained intact when placed in formalin. Histologic sectioning of the specimen showed the same type fibrin and platelet immeshed clot as was present in clots that formed under the more natural condition of gradual occlusion over 12–24 hours.

Anatomical

The postmortem examination of the hearts closely resembled the human condition. The helical coil wire was completely occluded with clot but the vessel both proximal and distal to the blockade was patent. While the clot was firm and did not break up when manipulated by forceps, it could be easily teased away from the arterial wall. After 8–10 days the magnesium alloy began to disintegrate but at no time was the intima of the coronary artery involved in the pathologic process. Microscopic examination of the clot revealed mostly fibrin and platelets in a haphazard arrangement which never demonstrated the concentric laminar appearance of slowly progressive thrombosis formation.

The myocardial infarction distal to the arterial occlusion involved the entire wall thickness with the wedge shaped distribution of that particular vessel. The animals dying early had only hemorrhagic changes of the myocardium while those sacrificed 10–14 days after infarction showed thinning of the ventricular wall plus scar formation which permitted the infarction to be easily demarcated from the viable surrounding myocardium.

DISCUSSION

Several authors have described methods for experimental production of acute myocardial infarction in the closed chest animals in order to attempt close resemblance of the human condition. Gensini et al. used microspheres to selectively occlude the left anterior branch, producing distal myocardial infarction with both hemodynamic and ECG changes.[5] More recently Lluch et al. have injected a single dose of metallic mercury into the circumflex branch to produce cardiogenic shock.[6] Both of these models approximate human myocardial infarction; however the multiple blockade of small peripheral vessels is anatomically different from the large vessel, gradually occlusive, process seen in the clinical condition. In 1968 Nakhjavan described a technique for insertion of a stainless steel cylinder into a branch of the left coronary artery.[7] Blood flow continued through the orifice for several hours if immediate clotting was prevented by anticoagulation. Significant arrhythmias, intra-ventricular conduction defects, and a 50% early mortality characterized this model. Hood, in 1970, described their results in a method of coronary occlusion which employed a balloon cuff device implanted about the coronary artery. They noted moderate left ventricular failure, a decline in cardiac output, and a 20% incidence of ventricular fibrillation.[8]

The thrombogenic properties of several metals has been recognized earlier and in 1951 Stone and Lord suggested the use of magnesium alloys threaded into saccular abdominal aneurysms to promote thrombosis and thereby prevent leakage or rupture.[9] Utilizing these observations, Blair et al. in 1964 proposed a model of experimental myocardial infarction using magnesium alloys to promote thrombus forma-

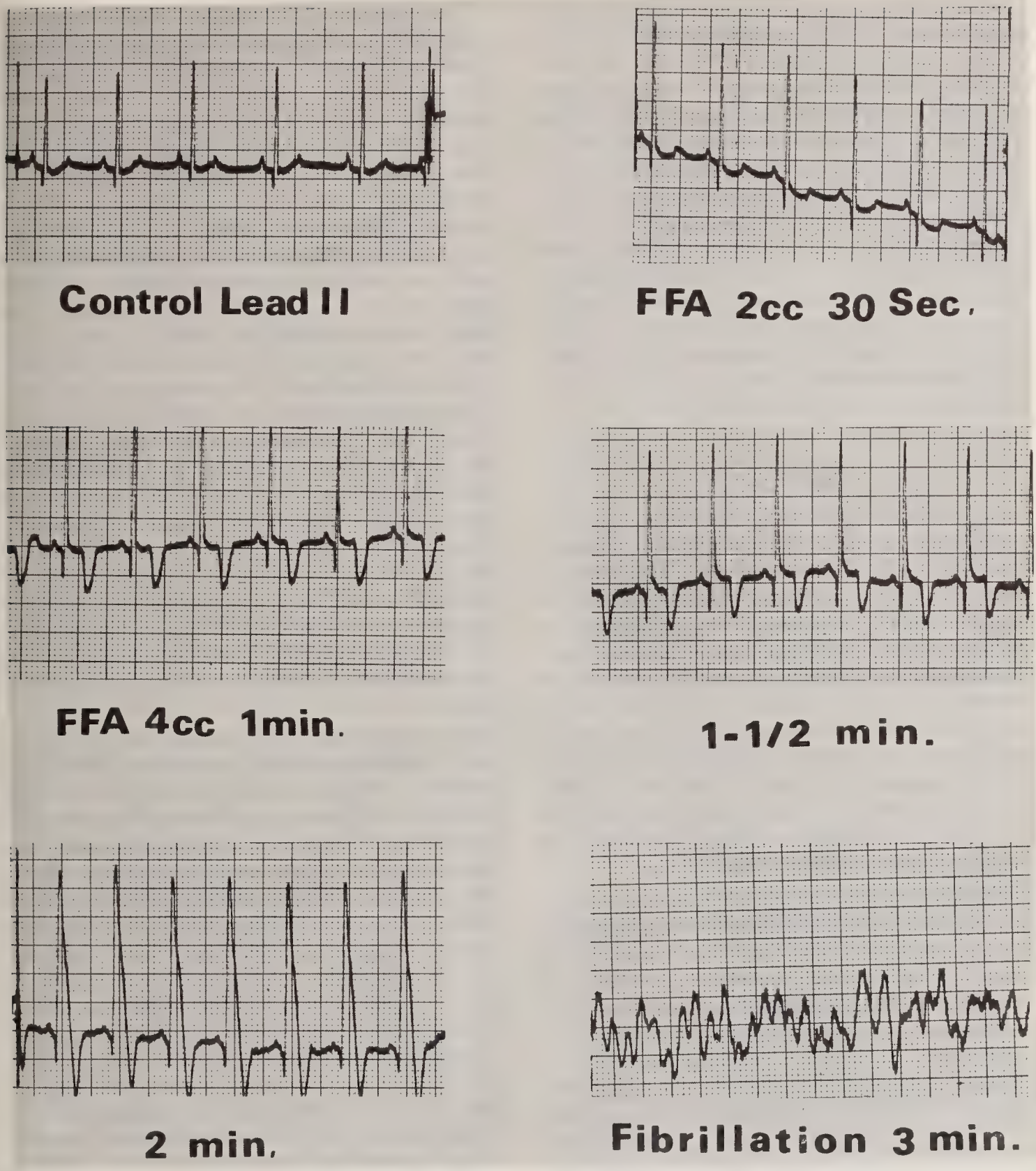


FIGURE 3.—Acute ischemic ST-T changes occurring immediately after injection of 0.1% palmitic acid. Acute cardiac dilatation and mechanical asystole were followed by a terminal ventricular fibrillation.

tion in the coronary artery.[10] At thoracotomy, a magnesium aluminum coil was threaded into the left anterior descending artery of dogs. Complete occlusion occurred in 5–7 days. This occlusion resembled the human process in that the intimal layer of the arterial wall was involved in the laminar thrombosis. Kezdi et al. reported that this myocardial infarction model was associated with a decrease in arterial blood pressure, cardiac output and maximum acceleration of aortic flow.[11] These hemodynamic parameters returned toward the normal in several days. The slowly developing arterial occlusion permitted collateral circulation to develop which decreased the resultant myocardial infarction size.

While the helical coil model of Blair resembled the human condition, a prolonged interval until complete occlusion occurred and the pericardiotomy with its resulting ECG changes were undesirable. Selective release of the helical coil wire into a branch of the left coronary artery under fluoroscopic control by the described catheter technique removed the need for an open-chest procedure. By this technique the occlusive process, instead of gradual thrombosis over several days, proceeds rapidly within 24 hours. The clot, while firmly attached to the wire, is not adherent to the intimal wall of the artery. Apparently injury to the arterial wall by wire penetration (Blair technique) results in gradual thrombosis while intra-arterial insertion develops rapid clotting.

The time of arterial obstruction could be altered by drugs which prolong the clotting mechanism. Single injections of Na heparin were effective in our model, whereas continuous anticoagulation with heparin was required by Nakhjavan et al. in their study to retard the occlusive process. The steel cylinder sleeve used in their study permits less flow, has a greater surface area exposed to the blood elements, and has a different thrombogenic quality than magnesium. The helical coil wire with its small surface area, nominal obstruction to flow, and slow tendency to clot requires little anticoagulation to prolong the time of obstruction. However immediate complete obstruction of the helical coil site can be achieved, when desired, by the use of long chain fatty acids.

The phenomena of rapidly induced intravascular thrombosis by fatty acids is well recognized and has been visualized in the living extracorporeal eye. Seaman et al.[12] demonstrated the deposition of platelet aggregates with fibrin on both arteries and veins. In fact, a 0.1% solution of sodium stearate may cause clotting in the syringe by precipitation of the contact activation phase of the water fall sequence of coagulation.[13] These platelet aggregates may occur in less than one minute, require calcium ions, and are not inhibited by heparin. This very rapid occlusion was noted in our study. The consequences of immediate free fatty acid induced occlusion were catastrophic and entirely different from the gradual process of thrombosis. Mechanical contractions immediately ceased, cardiac dilatation progressed rapidly and mechanical electrical dissociation was noted. Sinus rhythm and the hyperacute ischemic changes of the ST-T wave were present in the ECG for several seconds after absence of mechanical systole was noted on the fluoroscopic screen. Either ventricular fibrillation or electrical asystole, neither of which responded to resuscitative measures, was the terminal event.

The association of elevated free fatty acids, arrhythmias and a high incidence of death has been reported in patients with acute myocardial infarction.[14] The same authors (Oliver et al.) demonstrated that the elevation of plasma free fatty acids through activation of lipoprotein lipase by heparin was associated with serious ventricular arrhythmias in dogs with acute myocardial infarction.[15] More recently, Henderson et al. have studied the effects of free fatty acids on myocardial contractility during anoxia.[16] While free fatty acids had no significant effect on isolated rat muscle strips under normal oxygen pressure, both hypoxic and anoxic muscles had a gradual decrease of contractility to 30% of the control level. This decrease of force development could be partially restored by addition of calcium to the media, suggesting that the calcium available for the contractility process had been bound by the free fatty acid. These observations may partially explain the observed electrical-mechanical disassociation occurring in our model; the suddenly hypoxic heart with

an excess of free fatty acids and depression of muscle contractility undergoes rapid dilatation, complete pump failure, and terminal arrhythmias.

Cardiac arrhythmias, particularly those of ventricular origin, have been recognized in the experimental infarction in the dog since Harris noted the delayed development of ventricular ectopic rhythms.[17] In the studies of Berglund et al., hemodynamic measurements prior to the ventricular fibrillation have not shown any single pattern in the experimental coronary occlusion.[18] Primary fibrillation without pump failure occurred in 50%, gradual failure with terminal arrhythmia in 20%, and combined failure and dysrhythmia in 30% of the series. However, not all ventricular arrhythmias are precursors to ventricular fibrillation (at least in the experimental animal). Idioventricular tachycardia, complicating ligation of the anterior descending coronary artery in the dog, occurred in 48% of the study by Logic.[19] In no instance was spontaneous ventricular fibrillation noted, probably since idioventricular tachycardia is not associated with early, closely coupled extrasystoles. Joison at al., using the balloon cuff model of coronary occlusion, noted the development of a multifocal ventricular tachycardia 1–3 days after experimental dog myocardial infarction; only a small number of animals died of ventricular fibrillation and sinus rhythm was later restored.[20] These findings are similar to our results with the closed chest wire technique. Also the idioventricular tachycardia, unlike other ventricular arrhythmias, rarely causes hemodynamic embarrassment since loss of atrial drive rarely becomes significant.[21]

The hemodynamic changes in experimental myocardial infarction will vary depending upon the technique utilized and the total left ventricular mass involved. Selective embolization of the circumflex artery with 0.2 cc mercury produces a shock-like state and death of the animal within 48 hours. Misra et al. reported a decrease of 40% in the systemic blood pressure, 54% of cardiac output, and an increase of 28% in the heart rate with this technique.[22] The magnesium helical wire method of Blair was used by Kezdi et al. to produce gradual obstruction of the left anterior descending artery. This method resulted in a decrease in cardiac output of 15%, blood pressure of 12%, and a heart rate increase of 5%.[11] The present myocardial infarction model resulted in greater hemodynamic alterations (C.O. decrease 42%) than our previous open-chest model, but does not result in shock and early death as the Lluch mercury model. Return toward control values occurs in the hemodynamic variables over several days in hearts with 26% of the left ventricle involved in the infarction. This is similar to the experience of Hood et al. who demonstrated a decreased response to inotropic stimulation and increased left ventricular end-diastolic pressures in spite of normal hemodynamics four days after acute anterior descending ligation.[23]

SUMMARY

A small myocardial infarction while demonstrating significant hemodynamic deterioration is compatible with survival. The initial 48-hour mortality rate of 16% is similar to the more recent experience of coronary care units.[24] The hemodynamic and electrophysiologic similarities of this experimental infarction model to the human conditions are noteworthy. Gradual intracoronary occlusion in the awake intact animal, predictability of myocardial infarction, and the anatomical resemblance contribute to its usefulness in the study of drug effects on the electrical and hemodynamic alterations during infarction. Adaptation of this technique in the experimental laboratory should increase the knowledge of the pathophysiologic state existing with acute myocardial infarction.

REFERENCES

1. Agress, C. M., Rosenberg, M. S., Jacob, H. I., Bender, M. S., Schneidermen, A., and Clark, W. G. Protracted shock in closed chest dog following coronary embolization with graduated microspheres. Am. J. Physiol. 170:536–549, 1952.
2. Vineberg, A., Mahenti, B., and Litvan, J. Experimental gradual coronary artery constriction by Ameroid constrictors. Am. Heart J. 53:505, 1957.
3. Salazor, A. E. Experimental myocardial infarction, induction of coronary thrombosis in the intact closed chest dog. Circ. Res. 9:1351–1356, 1961.
4. Stanley, E. L. and Kezdi, P. Myocardial infarction model, serial physiologic measurements in

awake closed chest dogs. Presented at the 53rd Annual Meeting FASEB, April, 1969, Atlantic City. Abstract in Proceedings.

5. Gensini, G. G., Palacio, A., Buonenno, C., Kelly, A. E., and Muller, W. F. Super selective coronary occlusion under cinefluoroscopic control in experimental animals: Technique and Results. (Abst.) Cir. 34:III 108, 1966.
6. Lluch, S., Moguilevsky, H. C., Peitra, G., Shaffer, B., Hirsch, J. J., and Fishman, A. P. A reproducible model of cardiogenic shock in the dog. Circ. 39:205, 1969.
7. Nakhjavan, F. K., Shedrovilzky, H., and Goldberg, H. Experimental myocardial infarction in dogs—description of a closed chest technique. Cir. 38:777, 1968.
8. Hood, W. B., Jr., Joison, J., Kumar, R.,Katayoma, I., Neiman, R. S., and Norman, J. C. Experimental myocardial infarction. I. Production of left ventricular failure by gradual coronary occlusion in intact conscious dogs. Cardiovascular Res. 4:73–83, 1970.
9. Stone, Peter and Lord, S. W. An experimental study of the thrombogenic properties of magnesium and magnesium-aluminum wire in the dog's aorta. Surg. 30:987–993, 1951.
10. Blair, E., Nygren, E., and Cowley, A. A spiral wire technique for producing gradually occlusive coronary thrombosis. J. Thorac. Cardiovasc. Surg. 48:476–485, 1964.
11. Kezdi, P., Stanley, E. L., Marshall, W. J., and Kordenat, R. K. Aortic flow velocity and acceleration as an index of ventricular performance during myocardial infarction. Am. J. Med. Sci. 257:67–71, 1969.
12. Seaman, A. J., Hutcher, C. L., Moffat, C. A., and Hueber, B. E. Induced intra-vascular thromboembolic phenomena. Arch. Int. Med. 119:600–604, 1967.
13. Hoak, J. C., Warner, E. D., and Connor, W. E. Platelets, fatty acids and thrombosis. Circ. Res. 20:11, 1967.
14. Oliver, M. F., Kurien, V. A., and Greenwood, T. W. Relation between serum-free-fatty acids and arrhythmias and death after acute myocardial infarction. Lancet 1:710, 1968.
15. Kurien, V. A., Yates, P. A., and Oliver, M. F. Free fatty acids, heparin, and arrhythmias during experimental myocardial infarction. Lancet 2:185–187, 1969.
16. Henderson, A. H., Albert, M. B., Most, S., Parmley, W. W., Gorlen, R., and Sonnenblick, E. H. Depression of myocardial contractility in rats by free fatty acids during hypoxia. Circ. Res. 26:439–449, 1970.
17. Harris, A. S. Delayed development of ventricular ectopic rhythm following experimental coronary occlusion. Circ. 1:1318, 1950.
18. Berglund, Erie, Resnekxe, Leon, William-Olsson, Goran, and Ohrlund, Ann-Christine. Hemodynamic measurements prior to ventricular fibrillation or asystole following experimental myocardial infarction. Thorax 24:626–620, 1969.
19. Logic, J. R., Morrow, D. H., and Gaty, R. N. Idioventricular tachycardia complicating experimental myocardial infarction. Dis. Chest 56:477–480, 1969.
20. Joison, J., Kumar, R., Hood, W. B., Jr., and Norman, J. C. An implantable system for producing left ventricular failure for circulatory assist device evaluation. Trans. Amer. Soc. Artif. Int. Organs 15:417–422, 1969.
21. Schamroth, L. Idioventricular tachycardia (Editorial) Dis. Chest 56:466–468, 1969.
22. Misra, S. N., Stanley, E. L., and Kezdi, P. Hemodynamic determinants of cardiogenic shock. J. Surg. Oncol. 2(3):245–256, 1970.
23. Hood, W. B., McCarthey, B., and Lown, B. Myocardial infarction following coronary ligation in dogs. Circ. Res. 21:191, 1967.
24. Bloomfield, D. K., Slivka, J., Vossler, S., and Edelstein, J. Survival in acute myocardial infarction. Before and after the establishment of a coronary care unit. Dis. Chest. 57:224–229, 1970.

DISCUSSION

Chairman La Farge: Thank you very much, Dr. Stanley. Dr. Malinow?

M. R. Malinow, Beaverton, Oregon: I want to ask two questions. The first is: how did you dissolve palmitic acid to inject it? And the second is: how long did the animals remain heparinized with the dose you gave?

Dr. Stanley: I'll answer the second question first. We did not actually measure the time. We did draw blood from a few of the animals and put it into the tube half an hour to an hour later; by then the clotting time would be less than thirty minutes. So we did not measure Lee White clotting times in these animals at all. But judging from the tubes of blood which were removed within half an hour to an hour, I would assume that these animals were not heparinized for more than fiften to thirty minutes. I really believe that the reason why pretreatment with a small amount of heparin does work is that at the time of manipulation around the left coronary ostia you do abrade the endothelium a little, with probably a little release of fibrin or platelets; this is a critical time, because if there is too much trauma to the intima of the wall you do seem to have problems with the animals. And I think that pretreatment

with heparin only gets you over this short period of time.

I might mention another matter which is important here. Blair, in his original study, and we, in our work, found when using exactly the same type of magnesium alloy and inserting the coil through and actually traumatizing the coronary wall, it took several days for the occlusion to occur; histologically, however, it was a true thrombosis with lamina type construction. In our particular study when the arterial wall was not damaged at all while the coil was lying inside the lumen, occlusion occurred within twelve to twenty-four hours. It was not a thrombosis with lamina construction. Instead, it was actually a clotting type occlusion with a haphazard arrangement of platelets and fibrin.

As for the first question: it is quite difficult. The melting point of palmitic acid is, I believe, about 60° C. Originally, we tried to put in some sodium chloride and other salts to keep it in solution, or to dissolve it. This was not possible; so we finally heated up the distilled water solution to 60 to 70°. We added very small amounts of palmitic acid, and then constantly stirred it with a magnet. You end up with a slightly supersaturated solution, but as the temperature of the solution drops to 60, 55, and 50°, it does not precipitate. Accordingly, the actual solutions that we injected into these animals were in fact small amounts of a solution which was still heated up to 50 to 55° C.

DR. MALINOW: What happened to the dogs when you injected saline at 55°?

DR. STANLEY: Absolutely nothing happened at all. It is of interest that we also injected up to 20 cc. of the same free fatty acid solution without the helical coil wiring; the effects were quite different. Over a period of five to ten minutes there were some gradual ECG changes in some of the animals. When again injected with large amounts of the free fatty acid, the animals would demonstrate this gradual loss of mechanical systole with terminal arrhythmias after five to fifteen minutes. But the postmortem examination showed no major vessel, or even small vessel, occlusion in these animals. Specifically, very small amounts injected into a large vessel ischemic area seemed to have a profoundly different effect.

CHAIRMAN: I'd like to ask you a triple-headed question. This is a very nice model if you have a very high survival rate, such as 70% in eight or nine days which seems very good to me for coronary occlusion techniques. Do you consider longer term application? Do you suppose that the animals could go on living for, say, a month to provide a really long term follow-up? Have you tried any therapeutic maneuvers, and what do you foresee concerning the application of this technique in the artificial heart program?

DR. STANLEY: Yes, we let a few of them, included in this series, survive for up to a month's time. I think the survival rate is not due to the small occlusion only, but to the fact that we performed merely a small operation on the neck and closed that up; therefore we do not have an infection problem or similar problems. The animals that do survive for long periods of time are then sacrificed. The wire itself disintegrates after two weeks' time. The occlusion site recannulizes which I think others have demonstrated as well. In fact, after three weeks there is a significant recannulization and after a month or so, some of these animals showed a rather significant flow back through the vessel that was originally occluded. It is accordingly compatible with a long-term evaluation of the effect of small myocardial infarction. Finally, since this is a clotting or thrombotic mechanism, we have been interested in the study of various fibrinolytic and other agents in this particular model. Likewise, since a gradual occlusion over a period of several hours is involved, the development of collateral circulation is important. It is a fine model for that purpose, and we have studied various vasodilators and other drugs, medications or biochemical approaches so as to improve oxygen delivery to the heart itself. I therefore believe that it is a good model.

QUESTIONER: What gauge wire was used, and what effect did this have on the animal?

DR. STANLEY: The wire that we used is two hundredths of an inch in diameter. Originally, most of this work was done using a magnesium aluminum alloy which is very hard to come by, I might say. One company in Michigan was able to supply it for us. More recently we have gone

over to copper wire, and have pretreated with heparin, which seems to overcome this critical period. Some metals, such as iron, copper and similar metals will cause clotting within five to ten minutes. However, using a small-diameter wire and pretreating again with 4,000 units of heparin, we found that we can obtain an occlusion in two hours. This seems to be a very nice model and a fine material, to be used if you are particularly interested in fibrinolytic agents of one type or another. Magnesium is hard to find. In reply to your second question: you merely select dogs that have coronary arteries of about this size. In dogs weighing 15 to 20 kilograms, the wire fits around a 19-gauge needle. It works very well.

LEFT VENTRICULAR FAILURE AND CARDIOGENIC SHOCK FOLLOWING ACUTE MYOCARDIAL INFARCTION: A CORRELATION OF CLINICAL AND EXPERIMENTAL OBSERVATIONS

K. T. Weber, R. A. Ratshin, C. E. Rackley and R. O. Russell, Jr.*

Left ventricular failure and shock accompanying acute myocardial infarction (AMI) remain major therapeutic challenges. Experimental models have been developed to improve our understanding of the pathophysiology of these disorders; however, their validity has been open to question. In order to establish the experimental criteria necessary for an appropriate cardiac model, hemodynamic observations in 79 patients with AMI shock (45, Group A) or failure (34, Group B) are reported. No statistically significant difference was observed in heart rate (HR), peripheral vascular resistance (PVR), or left ventricular filling pressure (LVFP) between each group. Stroke index (SI), however, did reflect an important differentiation between failure and shock with a mean value of 18 cc/m^2 for Group A and 28 cc/m^2 for Group B. Cardiac index (CI) was 1.64 and 2.46 L/min/m^2, respectively. A composite literature review of AMI shock (153 patients), including this series, indicated the following mean values; HR, 102 beats/minute; SI, 15 cc/m^2; CI, 1.5 L/min/m^2; TPR, 2116 dynes/sec/cm^{-5}; and LVFP, 22 mm Hg.

Three experimental models that have been developed in conscious and anesthetized calves are described and include both slow (4–6 hours) and rapid (15–30 minutes) microsphere (6–14 micron diameter) embolization techniques of the left coronary artery, as well as controlled large vessel coronary occlusions. Persistent (3–28 days), dose-dependent degrees of ventricular impairment, at rest and during stress (angiotensin), associated with compensatory changes in ventricular wall thickness are seen for the slow microsphere infusion technique. Acute occlusions of the left anterior descending or circumflex coronary arteries resulted in acute ventricular failure without shock and a high incidence of fatal ventricular arrhythmias within 16 to 218 minutes. Rapid sphere injections, on the other hand, produced a stable degree of hemodynamic shock, with or without elevations in LVFP, and moderate elevations in PVR.

The criteria for failure and shock models are enumerated and comparisons with various experimental methods drawn.

*Department of Medicine University of Alabama, Birmingham, Alabama.

**The research upon which this publication is based was performed pursuant to the Medical Devices Applications Program Contract #PH 43-67-1418, N.I.H. Grant #HE 11,310; and the Myocardial Infarction Research Unit Program Contract #PH 43-67-1441, National Institutes of Health.

INTRODUCTION**

Left ventricular failure and cardiogenic shock accompanying acute myocardial infarction remain major therapeutic challenges. The inability of presently available medical therapeutics to improve survival has prompted the search for more aggressive interventions, such as infarctectomy, coronary bypass grafting and circulatory assist devices. In an attempt to improve our understanding of the pathophysiology, time course and therapeutic response of the ischemic heart during these states, a great deal of effort has been devoted to the development of an experimental analog.

However, before one can judge the adequacy and reliability of current ischemic heart models and evolve design criteria necessary for future preparations, a reasonable understanding of the clinical problem is necessary. Clearly, at present, we do not have all the answers on shock and failure and, in addition, obvious shortcomings always exist in equating experimental and clinical data. Aside from these inherent weaknesses, however, we feel that the present state of the art for the ischemic heart disease model can be assessed, but only after a careful review of pertinent and presently available clinical findings. The purpose of this paper will be to derive and describe experimental design criteria by reviewing the clinical and hemodynamic data in 79 patients, 45 with cardiogenic shock and 34 in failure without shock accompanying acute myocardial infarction, who were followed in our Myocardial Infarction Research Unit. The cor-

relation of these observation with several experimental preparations which we and others have utilized over the past several years will allow us to assess the present state of the art for experimental analogs.

METHODS

Clinical Data

Selection of patients: Seventy-nine patients with ventricular dysfunction complicating acute myocardial infarction were admitted to the Myocardial Infarction Research Unit (January, 1970 to January, 1972) and subsequently underwent hemodynamic evaluation. The diagnosis of acute myocardial infarction was established by an appropriate history and clinical picture in association with electrocardiographic evidence of either transmural or subendocardial infarction. The development of diagnostic Q waves and evolutionary changes in the ST segment and T waves and associated serial enzyme changes (CPK, SGOT and/or LDH) were required. Left bundle branch block (LBBB) was observed in five patients and was considered separately. All patients were studied within several hours of the onset of their symptoms and within 16 hours after the development of shock or failure. Informed consent was obtained in all patients. Pertinent information relating to the presence and duration of angina, congestive heart failure, previous myocardial infarction, and hypertension was obtained on each patient. The diagnosis of shock was made in 45 patients (Group A, cases 1–22 previously reported)[1] with a systolic blood pressure of 90 mmHg or less (or 80 mmHg decline from basal levels in known hypertensives) and confirmed by intra-arterial catherization and not secondary to arrhythmias, hypovolemia, pain or drug reactions. Clinical signs of shock (seen in 45 patients) included depressed mentation, peripheral vascular constriction (pallor, cool and clammy skin) and oliguria. The criteria for ventricular failure without shock included the presence of a ventricular gallop sound (S_3) and/or pulmonary rales. Thirty-four patients were included here (Group B).

Hemodynamics

Right heart and arterial or left heart catheterization was accomplished under fluoroscopic control using an image intensifier. Phasic and mean pressures were monitored with Statham P23Db manometers and were amplified and displayed on a multi-channel direct writing recorder (Honeywell). The zero reference was marked at the mid-chest level. Pulmonary arterial and left ventricular or central aortic pressures were measured in the 79 patients in whom hemodynamic studies were performed. Left ventricular filling pressure (LVFP) was either measured directly using left ventricular end diastolic pressure (LVEDP) or indirectly using pulmonary arterial end diastolic pressure (PAEDP). Cardiac outputs were determined by indicator dilution technique (Indocyanine green) using central sampling. The measurements of cardiac output were obtained in duplicate and were reproducible within 15%. Sympathomimetic therapy for shock was not withheld prior to hemodynamic study when clinically indicated; however, none of the patients studied were supported by these agents or digitalis at the time of the baseline studies.

Patency of the arterial and venous catheters was maintained with constant or bolus heparin infusions. Catheters remained in position for 24 to 48 hours for hemodynamic monitoring and cardiac output determinations.

The following calculations were made: left ventricular stroke and minute work (gram meters/meter^2 and kilogram meters/minute/meter^2, respectively) using mean ejection pressure, left ventricular filling pressure, stroke index and heart rate. Peripheral vascular resistance (PVR, in millimeters of mercury/liter/minute/meter^2 or units) was derived from mean ejection pressure and cardiac index.

Experimental Data

Holstein, Hereford, Charolais and Angus calves, four to twelve weeks of age and weighing 50 to 100 kg, were utilized for the experimental studies. The animals were maintained in metabolic cages or stalls with stanchions both pre- and postoperatively. The animals were

fasted for 24 to 48 hours prior to surgery and treated prophylactically with intramuscular penicillin. Following anesthesia using techniques previously reported,[2,3] the animal was intubated and ventilation carried out with a pressure-cycled respirator. Blood gas tensions and pH were checked periodically and maintained within the physiological range.[4]

The left chest was entered through the fifth intercostal space with the animal in a right lateral recumbent position. The following instrumentation, which has been previously described,[2] was implanted in both the acute and chronic groups of animals (see Fig. 1): Central aortic pressure catheter via the internal mammary artery; left ventricular implantable pressure transducer; electromagnetic flow probes placed around the aortic root and left main coronary artery or its branches; limb or direct epicardial ECG leads; and specialized instrumentation for inducing myocardial ischemia and infarction which are described below.

Microsphere technique—A series of 17 animals was utilized for the evaluation of myocardial ischemia and infarction following the injection of microspheres (6–14 micron diameter) into the left main coronary artery. Specifically, this included a group of nine animals[5] with a four- to six-hour microsphere infusion of varying dose level (4–5 mg/kg body weight) while in the conscious state. Following the microsphere infusion, the animals were evaluated over a period of 3 to 28 days. Angiotensin stress responses (2.5 mg angiotensin in 500 cc of saline) were also assessed during this time interval. At autopsy, the heart was weighed and left ventricular wall thickness measured. Another group of animals (8) were studied during open thoracotomy with similar doses of microspheres.[6] Here, however, the microsphere infusion was carried out over a period of 15 to 30 minutes.

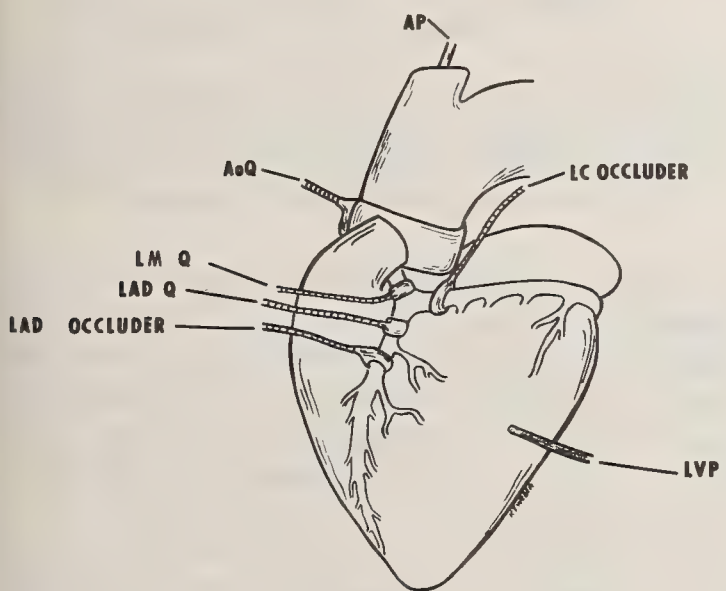


FIGURE 1.—Diagrammatic representation of instrumented calf heart. Included are an aortic root flow probe (AoQ); left main (LM) and left anterior descending (LAD) coronary artery flow probes; and left anterior descending and left circumflex (LC) coronary occluders. In addition, the left ventricular pressure transducer cable (LVP), and brachiocephalic pressure catheter (AP) are shown.

Coronary occluder technique—vascular occluders were positioned around the left anterior descending or circumflex coronaries in a group of five animals[6] studied in the conscious state.

Hemodynamics

All data were recorded on a direct writing multi-channel paper recorder (Beckman or Honeywell) as well as on analog tape. The tape was edited at a later date and various derivations made. These included the following: left ventricular systolic and end diastolic pressure; mean and pulsatile arterial pressure; stroke volume and cardiac output; left ventricular mean ejection rate from stroke volume and left ventricular ejection time; the maximum rate of pressure rise from the first derivative of left ventricular pressure; coronary blood flow; systolic time intervals including electromechanical systole (QS_2), left ventricular ejection time (LVET) and pre-ejection period (PEP); and peripheral vascular resistance (mm Hg/liter/minute) from mean arterial pressure and cardiac output.

Specifics concerning the surgical technique, postoperative maintenance, calibration, and monitoring of the implantable pressure transducer and other aspects of this instrumentation are being reported elsewhere in these Proceedings.[3] Normal resting hemodynamics for this calf preparation have also been previously documented.[2]

RESULTS

Clinical

Table I summarizes the clinical observations in the 79 patients which included 53 men and 26 women. The overall mean age was 59 years (29–83). The ratio of infarction location (anterior to inferior) was 27/15 and 20/12 for Groups A and B, respectively. Transmural and subendocardial infarctions were grouped together according to anterior or inferior position. Only five patients had left bundle branch block. Forty percent or more of all patients in Group A had angina, previous infarction, failure or hypertension; over 60% of these patients died. Those with previous infarction and failure had a 72% and 71% mortality, respectively. The overall mortality in Group A was 69%. In Group B, on the other hand, while over 36% had these accompanying complications, only three patients (9%) died.

The hemodynamic observations in each group included the following mean values: In Group A, heart rate (HR) 98 (60–149 beats/minute); stroke index (SI) 18 (7–39 cc/m^2); cardiac index (CI) 1.64 (0.6–3.1 L/min/m^2); peripheral vascular resistance (PVR) 60.5 (19–136 mmHg/L/min/m^2 or units); and LVFP 25 (2–60 mmHg). Mean PVR was 49 units in survivors and 70 units in nonsurvivors (Fig. 2). Depicted in Figure 3 is a hemodynamic subgrouping of shock patients based on cardiac index and LVFP. Twenty-five patients with anterior or inferior infarction, CI less than 2.1 and a LVFP greater than 15, had a 100% mortality.

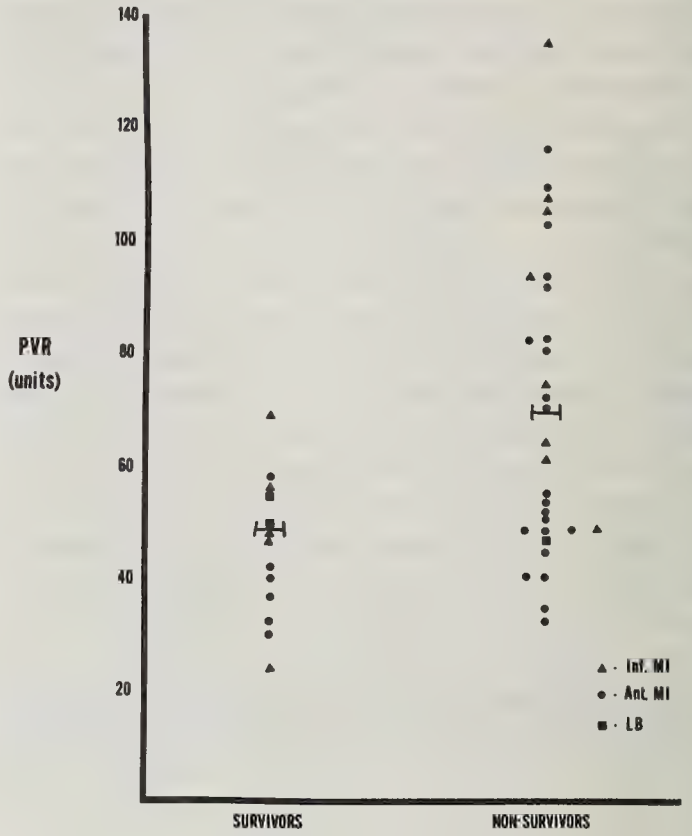


FIGURE 2.—Peripheral vascular resistance (mmH/L/min/m^2 or units) plotted for survivors and nonsurvivors in Group A (shock). Mean PVR for survivors is indicated at 48.4, and at 70.4 for non-survivors.

TABLE I.—*Clinical Data for Patients With Cardiogenic Shock and Failure Following Acute Infarction*

	Shock		Failure	
	No./45 (%)	% Mortality	No./34 (%)	% Mortality
Sex				
Male	31/45 (69%)	58%	22/34 (65%)	5%
Female	14/45 (31%)	7%	12/34 (35%)	17%
Age				
Mean	59		58	
Range	29–75		36–83	
Past medical history				
Angina	24/45 (53%)	66%	25/34 (74%)	8%
Previous infarction	18/45 (40%)	72%	14/34 (41%)	0%
Congestive failure	21/45 (47%)	71%	12/34 (35%)	16%
Hypertension	21/45 (47%)	61%	15/34 (33%)	20%
Transmural infarction				
Anterior	27/45 (60%)	82%	20/34 (58%)	10%
Inferior	15/45 (33%)	53%	12/34 (27%)	0%
Subendocardial infarction				
Anterior	1/45 (2%)	0%	9/34 (26%)	11%
Inferior	0/45 (0%)	0%	4/45 (12%)	0%
Left bundle branch block	3/45 (7%)	33%	2/34 (6%)	50%
Overall mortality	31/45	69%	3/34	9%

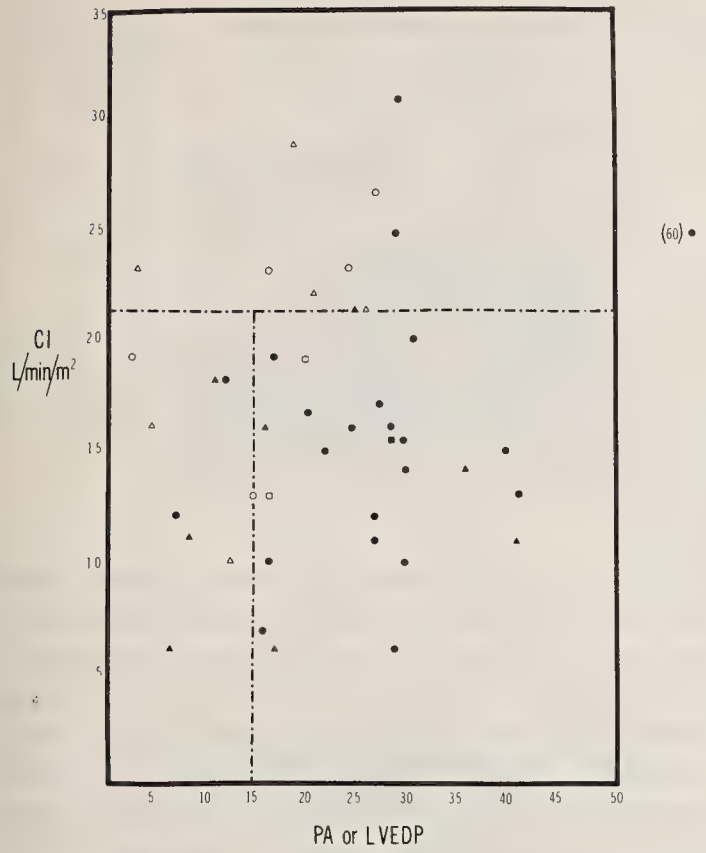


FIGURE 3.—A plot of cardiac index versus left ventricular filling pressure (mmHg expressed by pulmonary artery or left ventricular end diastolic pressure). The open symbols represent survivors and the closed symbols represent those patients which died. Triangles are representative of those patients with inferior myocardial infarction; circles represent those with anterior myocardial infarction; and squares represent those patients having left bundle branch block.

When three patients with LBBB were included, the mortality was 93%. In six patients with CI less than 2.1 and LVFP less than or equal to 15, 66% died. The four patients with CI greater than or equal to 2.1 regardless of LVFP had only a 36% mortality.

In Group B, mean HR was 94 (56–127); SI 28 (12–54); CI 2.46 (1.2–4.1); PVR 55.4 (25–129) and LVFP 20 (2–50). The mean hemodynamic values seen in Group B are similar to the findings in Group A with the exception of SI, which is substantially lower in Group A. This represents an approximate 60% and 38% reduction from normal (45 cc/m²) in SI for Groups A and B, respectively. Accompanying medical complications (angina, previous infarction, congestive failure and hypertension) did not influence the presenting hemodynamics in Group A or B.

Experimental

The results of the slow (4- to 6-hour) microsphere infusion method[5] in nine animals are shown in Table II. In the low dose group (animals 1 through 4; 4 mg/kg body weight) the primary hemodynamic abnormality at rest was the persistently elevated LVEDP. Flow and pressure characteristics of the heart were otherwise generally within the limits of normal for these animals.[2] The high dose group had several abnormal findings at rest including reductions in stroke volume, cardiac output, mean injection rate and rate of LV pressure rise. LVEDP was elevated chronically as in Group I. During angiotensin stress (Fig. 4), both groups demonstrated abnormal responses. This was particularly evident in Group II. Heart weight and wall thickness were increased 65% and 45%, respectively, above normal[2] for both groups.

Of particular note with this technique of slow microsphere embolization is the lack of ventricular arrhythmias during the infusion period. All animals, except 3 and 7, were electively sacrificed. These two animals died at three days postinjection with thrombosis of the left main coronary artery surrounding the infusion cannula. The histopathology in all animals demonstrated widespread cellular death and injury throughout the left ventricle at various states of development.[5]

Those animals receiving similar doses of microspheres (also 6–14 micron in diameter) but infused over 15 to 30 minutes, demonstrated a totally different response. In this series, stable hemodynamic shock was dominant with or without elevations in LVEDP. The average results[6] are presented in Table III. In addition, three of the seven animals died with irreversible ventricular fibrillation. Two others had electromechanical dissociation unresponsive to pharmacological support.

Major vessel occlusion representing 25% to 40% reduction of left main coronary flow in conscious animals resulted in hemodynamic and

TABLE II.—*Chronic Hemodynamic Data with Subacute Microsphere Injection*

Animal	Baseline	Days postinjection 1	3	5	10	14	21	28
Average (Group I; 4 mg/kg)								
LVP	132/9	118/15	115/17	117/14	124/22	114/20	132/19	
dp/dt	2217	1882	1763	1803	1947	1993	2120	
SV	87	77	75	76	83	81	64	
CO	8.3	8.3	8.4	8.3	8.9	8.8	7.9	
MER	438	398	388	374	418	382	410	271
Heart weight: 983 gm (960–1240); LV wall thickness: 2.8 cm (2.4–3.1).								
Average (Group II; 5 mg/kg)								
LVP	131/8	110/17	114/20	106/19	111/21	104/20	111/20	104/19
dp/dt	1958	1532	1538	1373	1452	1290	1450	1600
SV	81	51	55	52	59	56	50	51
CO	8.2	6.8	6.3	5.8	6.5	6.2	6.4	5.2
MER	266	302	339	274	321	299	381	
Heart weight: 967 gm (700–1550); LV wall thickness: 2.9 cm (2.6–3.0).								

clinical failure unaccompanied by shock (see Table IV). LVEDP in these animals ranged from 18 to 31 mmHg. In addition, all animals died with irreversible ventricular fibrillation within 16 to 218 minutes of occlusion.[6]

DISCUSSION

The primary intent of experimental ischemic heart models is to characterize ventricular dysfunction during various stages and degrees of ischemia and infarction and to then assess the ability of therapeutic interventions to alter and improve on these observed abnormalities. The design criteria for models representing the clinical spectrum of failure and shock will be presented below.

Ventricular dysfunction following acute myocardial infarction is variable. Shock accompanying acute infarction, and unrelated to arrhythmias, hypovolemia, pain or drug reaction, represents one end of the spectrum. The high mortality (66 to 100%) accompanying shock makes this entity the major therapeutic challenge in the ischemic heart disease area. Table V depicts the hemodynamic observations in shock reported by several investigators.[7–14] In addition, it has become clear that within shock, several hemodynamic subgroups may be identified and thereby provide pertinent prognostic and therapeutic information. The hemodynamic criteria for the experimental model which represents the clinical variant of acute myocardial infarction shock requiring the greatest research effort, therefore, are: (a) systolic pressure of

TABLE III.—*Cardiogenic Shock Following Rapid Microsphere Infusion*

	HR beats/min	LVP mmHg	AP mmHg	SV cc	CO L/min	TPR mmHg/ L/min
Basal						
Average	125	115/6	115/87	74	8.5	12.7
Range	76–162	95/2–125/10	95/65–125/95	51–100	6.5–11.0	10.8–16.3
Shock (37–85 min.)						
Average	118	71/16	71/48	37	3.7	14.2
Range	85–160	45/6–95/38	45/25–95/70	12–65	1.3–5.8	10.2–21.4

TABLE IV.—*Failure Following Large Vessel Occlusion in Conscious Animals*

	HR beats/min	LVP mmHg	AP mmHg	SV cc	CO L/min	TPR mmHg/ L/min
Basal						
Average	97	136/7	136/90	81	7.8	11.7
Range	75–112	118/2–165/10	118/70–165/105	72–97	5.8–8.6	10.2–13.0
Failure (10–203 min.)						
Average	107	118/22	118/81	57	6.1	13.0
Range	101–110	100/18–135/31	118/85–135/100	50–61	5.5–6.6	10.1–14.9

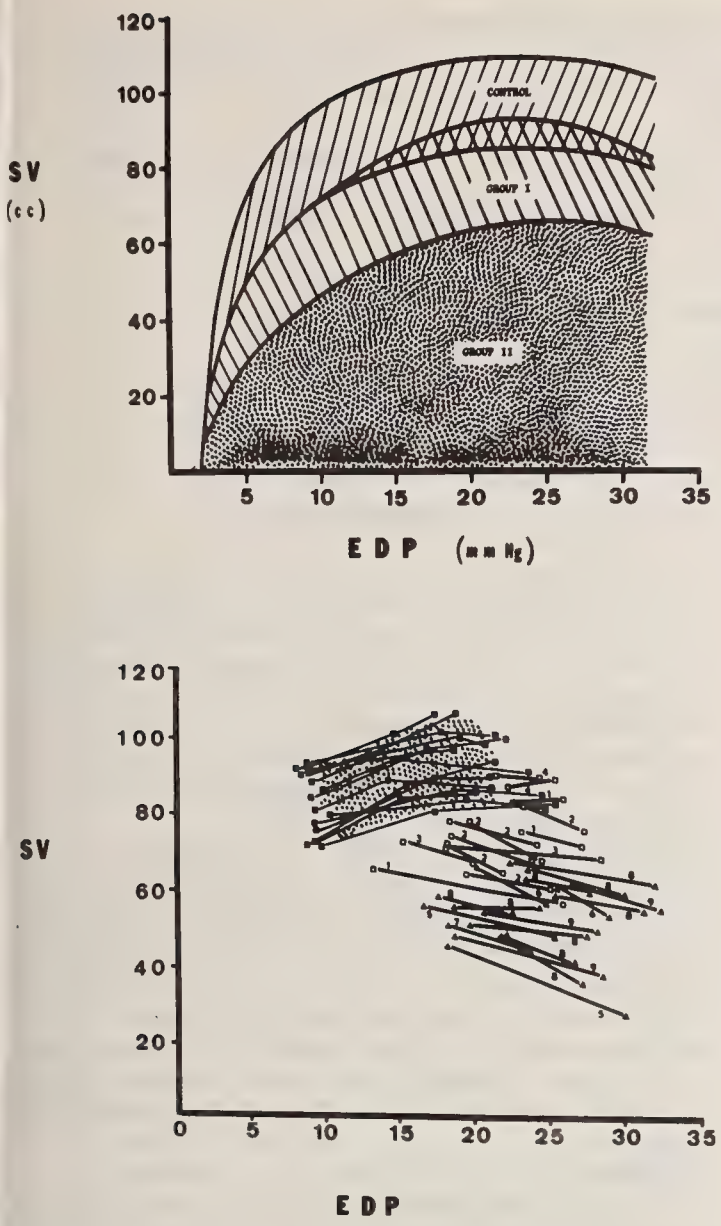


FIGURE 4.—Angiotensin stress curves following subacute microsphere infusion. The control observations for each animal are shown in the speckled area. The low (Group I) and high (Group II) dose groups are also represented.

90 mmHg or less and an elevated LVEDP of 15 mmHg or more; (b) reductions in CI of 40% or more; (c) an elevated PVR; and (d) a moderate tachycardia. In addition to these hemodynamic features, the preparation should be free of complicating ventricular arrhythmias which make successful data collection and interpretation difficult. The shortcomings of anesthesia and thoracotomy need only be cited for completeness. The percent of myocardial damage or infarction seen at autopsy in patients with shock has been reported as 40% to 60%.[15–17] Clearly, to experimentally evaluate an aggressive intervention such as the intra-aortic balloon in an animal model simulating the low mortality shock subgroup (CI greater than 2.1) would tend to lead to falsely optimistic conclusions.[18–19] Several experimental cardiogenic shock preparations which have appeared in the literature are listed in Table VI.[20–24]

Ventricular failure (without shock) in the ischemic heart is another major area of concern. Here, chronicity or the time period following infarction and the amount of compensatory change (i.e., wall thickness, chamber dilatation and increased rate of viable fiber shortening) is important in determining the level of impairment. The influence of these changes has been clearly demonstrated both clinically[25–27] and experimentally.[28–33] Also, the response to pharmacological agents, such as digitalis, will be variable for these reasons. Design criteria for a failure model would appear to include (a) reductions in coronary flow at a subacute rate[6,34] so as to permit coronary hyperemia and electromechanical stability during the induction period; (b) persistent elevations in LVEDP; (c)

TABLE V.—*Published Hemodynamic Data In Patients With Cardiogenic Shock*

Author	No. of Pts.	HR beats/min	SI cc/m²	CI L/min/m²	TPR dyne-sec-cm⁻⁵	LVFP mmHg
Scheidt	19	109	11	1.1	2600	22*
Smith	14	98	17	1.6	1586	—
Weil	20	95	14	1.3	2042	—
Gunnar	12	101	13	1.3	1688	—
Bradley	5	102	17	1.8	1448	21
Mueller	18	102	16	1.4	1575	—
Cohn	14	101	18	1.8	2386	23
MacKenzie	6	108	15	1.7	3016	—
Weber	45	98	18	1.6	2704	25
Average		102	15	1.5	2116	22

*7 pts.; — = Not reported.

TABLE VI.—*Published Data On Experimental Cardiogenic Shock*

Author	Animals	HR	SV	CO	TPR	LVEDP
Agress	12	146 (±)	−38%	−39%	±	—
Goldfarb	18	128 (±4)	—	−44%	+61% (±21)	23.6 (±3)
Lluch	17	174 ±7 (+27%)	—	−53%	+41%	7
Cronin	10	140 (−6%)	−55%	−58.5%	+42%	5.5
Kuhn	28	125 (−13%)	−26.5%	−35%	±	±
Weber	7	118 (±)	−50%	−50%	+11%	16

— = Not reported. ± = No change.

controllable and graded reductions in CI, SI and MER at rest or during stress; (d) freedom from fatal arrhythmias to permit chronicity; and (e) appropriate, though oftentimes inadequate, compensatory changes in the myocardium and coronary collateral bed. We believe this has been accomplished by Hood et al.[28–32] in their conscious dog preparation using single or multiple vascular occluders and the subacute microsphere technique.[5]

SUMMARY

The clinical spectrum of myocardial impairment or dysfunction seen with ischemic heart disease is quite broad and dependent on the time of observation in the overall course of the disease and the existing compensatory changes of the myocardium and coronary circulation. These factors are not only important in designing an experimental cardiac model but also in its overall assessment with various therapeutic interventions. Design criteria for shock and failure models following acute myocardial infarction are presented and the current state of the art assessed.

ACKNOWLEDGEMENT

Drs. Frank W. Hastings and T. Joseph Reeves, whose spirited and probing comments provided many creative insights during the course of the investigations which are herein reviewed, I gratefully acknowledge. Miss Susan Green is also acknowledged for her dedicated secretarial assistance.

REFERENCES

1. RATSHIN, R. A., RACKLEY, C. E., and RUSSELL, JR., R. O. Hemodynamic evaluation of left ventricular function in shock complicating myocardial infarction. Circ., 45:127–139, 1972.
2. WEBER, K. T., DENNISON, B. H., FUQUA, J. M., SPEAKER, D. M., and HASTINGS, F. W. Hemodynamic measurements in unanesthetized calves. J. Surg. Res., 11:383–389, 1971.
3. WEBER, K. T., JANICKI, J. S., WALKER, A. A., and DENNISON, B. H. The conscious calf preparation: Technical aspects of surgery, animal care, implanted instrumentation and monitoring for cardiovascular data acquisition. *Research Animals in Medicine*. G.P.O., Washington, D.C., 10–2, 1972.
4. DONAWICK, W. J. and BAUE, A. E. Blood gases, acid-base balance, and alveolar-arterial oxygen gradient in calves. Am. J. Vet. Res., 29:561–567, 1968.
5. WEBER, K. T., MALININ, T. I., DENNISON, B. H., FUQUA, J. M., Speaker, D. M. and HASTINGS, F. W. Experimental myocardial ischemia and infarction: Production of diffuse myocardial lesions in unanesthetized calves. Amer. J. Card., 29:793–802, 1972.
6. WEBER, K. T., MALININ, T. I., HECK, F. J. and DENNISON, B. H. Experimental production of vascular and microvascular myocardial ischemia and infarction: The effects of different rates and degrees of coronary flow reduction in calves. *Reversability of Cellular Injury due to Inadequate Perfusion*. C. C. Thomas, Springfield, Illinois, 266–287, 1972.
7. SCHEIDT, S., ASCHEIM, R., and KILLIP, T. Shock after acute myocardial infarction. A clinical and hemodynamic profile. Amer. J. Card., 26:556–564, 1970.
8. SMITH, H. J., ORIOL, A., MORCH, J., and MCGREGOR, M. Hemodynamic studies in cardiogenic shock. Treatment with isoproterenol and metaraminol. Circ., 35:1084–1091, 1967.
9. WEIL, M. H. and SHUBIN, H. Shock following acute myocardial infarction. Current understanding of hemodynamic mechanisms. Prog. in Cardiovas. Dis., 11:1–17, 1968.

10. Gunnar, R. M., Cruz, A., Boswell, J., Pietras, R. J., and Tobin, J. R. Myocardial infarction with shock. Hemodynamic studies and results of therapy. Circ., 33:753–762, 1966.
11. Bradley, R. D., Jenkins, B. S., and Branthwaite, M. A. The influence of atrial pressure on cardiac performance following myocardial infarction complicated by shock. Circ., 52:827–837, 1970.
12. Mueller, H., Ayres, S. M., Gregory, J. J., Giannelli, S., and Grace, W. J. Hemodynamics, coronary blood flow and myocardial metabolism in coronary shock: Response to 1-Norepinephrine and Isoproterenol. J. Clin. Invest., 49:1885–1902, 1970.
13. Cohn, J. N., Tristani, F. E., and Khatri, I. M. Studies in clinical shock and hypotension VI. Relationship between left and right ventricular function. J. Clin. Invest., 48:2008–2018, 1969.
14. MacKenzie, G. J., Taylor, S. H., Flenley, D. C., McDonald, A. H., Staunton, H. P., and Donald, K. W. Circulatory and respiratory studies in myocardial infarction and cardiogenic shock. The Lancet, 2:825–832, 1964.
15. Scheidt, S., Alonso, D. R., Post, M. R., and Killip, T. Quantification of myocardial damage in cardiogenic shock. Circulation 44(Suppl. II): II–46, 1971.
16. Lewis, R. K., Buettner, L. E., Massing, G. K., Russell, R. O., and Rackley, C. E. Clinical pathological correlations in acute myocardial infarction with and without shock. Circulation 44(Suppl.II): II–194, 1971.
17. Page, D. L., Caulfield, J. B., Kastor, J. A., DeSanctis, R. W., and Sanders, C. A. Myocardial changes associated with cardiogenic shock. New Eng. J. Med., 285:133–137, 1971.
18. Fleming, W. and Aaby, G. V. A comparative study of arterio-arterial and intra-aortic balloon counterpulsation in the therapy of cardiogenic shock. J. Thor. Cardiovas. Surg., 60:818–828, 1970.
19. Feola, M., Kennedy, J. H., Norman, N. A., and Haiderer, O. Intra-aortic balloon pumping at different levels of experimental acute left ventricular failure (IABP). Artif. Heart Program Conf., 637–657, June, 1969.
20. Agress, C. M., Glassner, H. F., Binder, M. J., and Fields, J. Hemodynamic measurements in experimental coronary shock. J. App. Physio., 10:469–475, 1957.
21. Goldfarb, D. and Gott, V. L. Cardiovascular alterations during declining and steady-state low cardiac output secondary to coronary insufficiency in the dog. J. Thor. and Cardiovas. Surg., 56: 578–589, 1968.
22. Lluch, S., Moguilevsky, H. C., Pietra, G., Shaffer, A. B., Hirsch, L. J., and Fishman, A. P. A reproducible model of cardiogenic shock in the dog. Circ., 39:205–217, 1969.
23. Cronin, R. F. P. and Zsoter, T. Hemodynamic effects of rapid digitalization in experimental cardiogenic shock. Amer. Heart J., 69:233–240, 1965.
24. Kuhn, L. A., Kline, H. J., Marana, A. J., Hamby, R. I., Cestero, J., Cohn, L. J., Weinrauch, H., and Berger, M. Mechanical increase of vascular resistance in experimental myocardial infarction with shock. Circ. Res., 19:1086–1096, 1966.
25. Baxley, W. A., Jones, W. B. and Dodge, H. T. Left ventricular anatomical and functional abnormalities in chronic post-infarction heart failure. Annals Int. Med., 74:499–508, 1972.
26. Saltups, A., McCallister, B. D., Hallermann, F. J., Wallace, R. B., Smith, R. E., and Frye, R. L. Left ventricular hemodynamics in patients with coronary artery disease and in normal subjects. Amer. J. Med., 50:8–19, 1971.
27. Rackley, C. E., Davis, H., Baxley, W. A., Jones, W. B., and Dodge, H. T. Left ventricular chamber volume, mass and function in severe coronary artery disease. Circ., 41:605–613, 1970.
28. Hood, W. B., McCarthy, B., and Lown, B. Myocardial infarction following coronary ligation in dogs Hemodynamic effects of isoproterenol and acetylstrophanthidin. Circ. Res. 21:191–199, 1967.
29. Kumar, R., Hood, W. B., Joison, J., Norman, J. C., and Abelmann, W. H. Experimental myocardial infarction II. Acute depression and subsequent recovery of left ventricular function: Serial measurements in intact conscious dogs. J. Clin. Invest., 49:55–62, 1970.
30. Hood, W. B. Experimental myocardial infarction III. Recovery of left ventricular function in the healing phase. Contribution of increased fiber shortening in noninfarcted myocardium. Amer. Heart J., 79:531–538, 1970.
31. Hood, W. B., Joison, J., Kumar, R., Katayama, I., Neiman, R. S., and Norman, J. C. Experimental myocardial infarction. I. Production of left ventricular failure by gradual coronary occlusion in intact conscious dogs. Cardiovas. Res., 4:73–83, 1970.
32. Hood, W. B., Kumar, R., Joison, J., and Norman, J. C. Experimental myocardial infarction. V. Reaction to impaired circumflex flow in the presence of estabblished anterior myocardial infarction in intact conscious dogs. Amer. J. Card., 26:355–364, 1970.
33. Weisse, A. B., Saffa, R. S., Levinson, G. E., Jacobson, W. W., and Regan, T. J. Left ventricular function during the early and late stages of scar formation following experimental myocardial infarction. Amer. Heart J., 79:370–383, 1970.
34. Walston, A., Donald, B., Hackel, E., and Harvey, E. Acute coronary occlusion and the "power failure" syndrome. Amer. Heart J., 79:613–619, 1970.

DISCUSSION

Chairman Kezdi: Dr. Weber, I agree with everything you said except concerning the systolic blood pressure. We know, for instance,

that different animals have different pressures; that in dogs, even the arresting pressure is higher than in humans. I don't know whether you can rely on systolic blood pressure as a criteria. I certainly agree with everything else you say about blood pressure.

K. T. WEBER, JR.: I must admit that the majority of the conscious animals that we have experimented with were calves. Their blood pressure, however, is within the range seen clinically for humans as we have previously reported. I understand from the conscious canine model series in the literature, that one does not see the hypertension and tachycardia which accompanies barbiturate anesthesia and thoracotomy. This may likewise influence the total peripheral resistance changes which are seen in some of the cardiac models, namely drops in peripheral resistance resulting in "warm" cardiogenic shock rather than the typical "cold" type of shock.

CHAIRMAN: The left ventricle end diastolic pressures are certainly very important: unless you have these, you probably have a hypovolemic animal. This is one of the important points when you should know whether or not you have a hypovolemic animal.

R. E. CLARK, Washington University, St. Louis, Mo.: I rise to emphasize Dr. Weber's figures on his patients. When the index was less than 2.1 liters per minute per meter squared, the mortality rate was very high. Most of our patients do not have the availability of the left ventricular pressure, particularly when we are concerned with the low pump syndrome following open heart surgery. We frequently do not have a left atrial pressure, and we are left with arterial pressure. Although we need more patients for a complete statistical anlysis, I think we have been able to document reasonably that, using a method of simultaneous dye and thermal cardiac output, the approximate cutoff in patients is at about 2 liters per minute. With an index of 2 liters per minute per meter squared, in our operative patients, we get a mortality rate of nearly 100%. Accordingly, at least the clinical data in surgery patients seems to correlate with the data you get in calves and in your myocardial infarction patients.

M. W. OSBORNE, Hoffman-Laroche, Inc., Nutley, N.J.: Could you describe the angiotensin stress test briefly, please. I am not acquainted with it.

K. T. WEBER: Yes. The angiotensin stress response was reported by Drs. Ross and Braunwald some years ago; it involves the intravenous administration of angiotensin or Hypertensin® to elevate systemic resistance (afterload) and evaluate ventricular function. The drip rate can be controlled and you can easily control the pressure level against which the heart has to pump. It is possible to analyze peak systolic pressures all the way up to 200 mm of mercury or more, if you decide to do so. Another attractive feature of this stress test is that the angiotensin effect does not persist for more than five minutes.

W. ERLICH: (Question: partially inaudible, concerns the survival rate and the compensating mechanism.)

K. T. WEBER: I agree that at first the peripheral bed responds to inadequate profusion with vasoconstriction. However, the choice of pharmacological vasodilators versus vasoconstrictors in these patients is not difficult. When we're dealing with blood pressures of 90 mm of mercury or less to begin with and cardiac indexes of less than 2.1 (and often as low as 0.6) it is difficult to justify the use of a vasodilator. Coronary perfusion pressure is already critically reduced. The major concern in infarction shock should be with the heart and not the periphery.

We are currently attempting to assess, with three-dimensional analysis, the influence of peripheral resistance, filling pressure, and cardiac index in predicting both prognostic and therapeutic hemodynamics sub-groups.

PROTEASES AND CHEMICAL MEDIATORS IN ACUTE CORONARY OCCLUSION IN THE DOG

H. J. Wilkens, R. Steger and N. Back*

The possible significance of protease activation and release of vasoactive mediators in the pathogenesis of acute myocardial infarction following coronary occlusion in the unanesthetized dog was studied. Dogs were prepared under anesthesia with a loose ligature around the anterior descending branch of the left coronary artery. The ends of the ligature were exteriorized through the chest wall, and both the pericardial sac and chest wall closed. After a 7-day recovery period, the ligatures were tightened suddenly and both physiologic and biochemical parameters studied at frequent intervals prior to and during the acute phase of the occlusion and for 6–8 days thereafter. In all, 14 dogs have been studied to date; 7 saline treated control dogs and 7 dogs that received a proteolytic enzyme inhibitor, TRASYLOL, 50,000 units/kg intravenously, just prior to coronary occlusion. Three control dogs died within 24 hours, while 2 drug-treated dogs died during the same time period. Control dogs evidenced more pain at the time of occlusion. Hematocrit values were higher in the control dogs from the 24-hour period. While prothrombin levels were decreased in both groups of dogs, Lee White clotting times were prolonged in the control dogs. A fibrinogen increase noted in the control dogs did not occur in the drug-treated dogs until 24 hours after occlusion. Initially euglobulin clot lysis times were not altered significantly but were shortened at 30 minutes in both groups. No blood kinin activity was detected in either group, although kininase activity was elevated in the control group. Kininogen decreases were evident in both groups from the 24-hour time period. These initial data encourage further study with this experimental model system.

INTRODUCTION**

Present evidence supports the concept that severe myocardial ischemia can cause ventricular arrhythmias leading to sudden death or serious pump failure.[1] Alterations in carbohydrates, lipid, and protein metabolism, as well as changes in intracellular enzyme systems, have been reported in the ischemic state.[2] Increased lactic acid levels have been demonstrated in coronary sinus blood within seconds after coronary artery ligation.[2,3] This lowered pH provides a suitable milieu for activation of lysosomal proteases that are released by cell wall changes and autolysis.

While the significance of these intracellular enzymes in the myocardial response to ischemia remains uncertain, their involvement in the pathologic process of infarction growth may be considered. In addition, recent studies have shown that only a slight lowering of blood pH results in the activation of plasma proteases and subsequent release of potent vasopeptides,[4] in accordance with the scheme seen in Fig. 1. The interrelationship between the fibrinolysin and vasoactive polypeptide systems (Fig. 2) has been reviewed.[5] Evidence supports the participation of kinins in the mechanism of spontaneous pain during myocardial ischemia, and that in the initial phase of myocardial infarction, the kinins formed by protease action participate in the mechanism of collapse and shock.[6,7] Bassange et al.[8] have identified a bradykinin potentiating peptide active in the coronary circulation following kinin administration.

The present studies were undertaken to provide a detailed integrated account of the role the protease and vasopeptide systems might play in the clinical sequence of acute myocardial ischemia. Use of protease inhibitors provides an additional tool for clarifying the role of proteases, and may yield information of possible therapeutic significance.

METHODS

Acute Coronary Occlusion

Fourteen healthy dogs, anesthetized with intravenous sodium pentobarbital, 35 mg/kg, were intubated to facilitate artificial respira-

* Department of Biochemical Pharmacology, School of Pharmacy, State University of New York, Buffalo, New York.

** Supported in part by USPHS Grants #HE-03487 and #HE-11492.

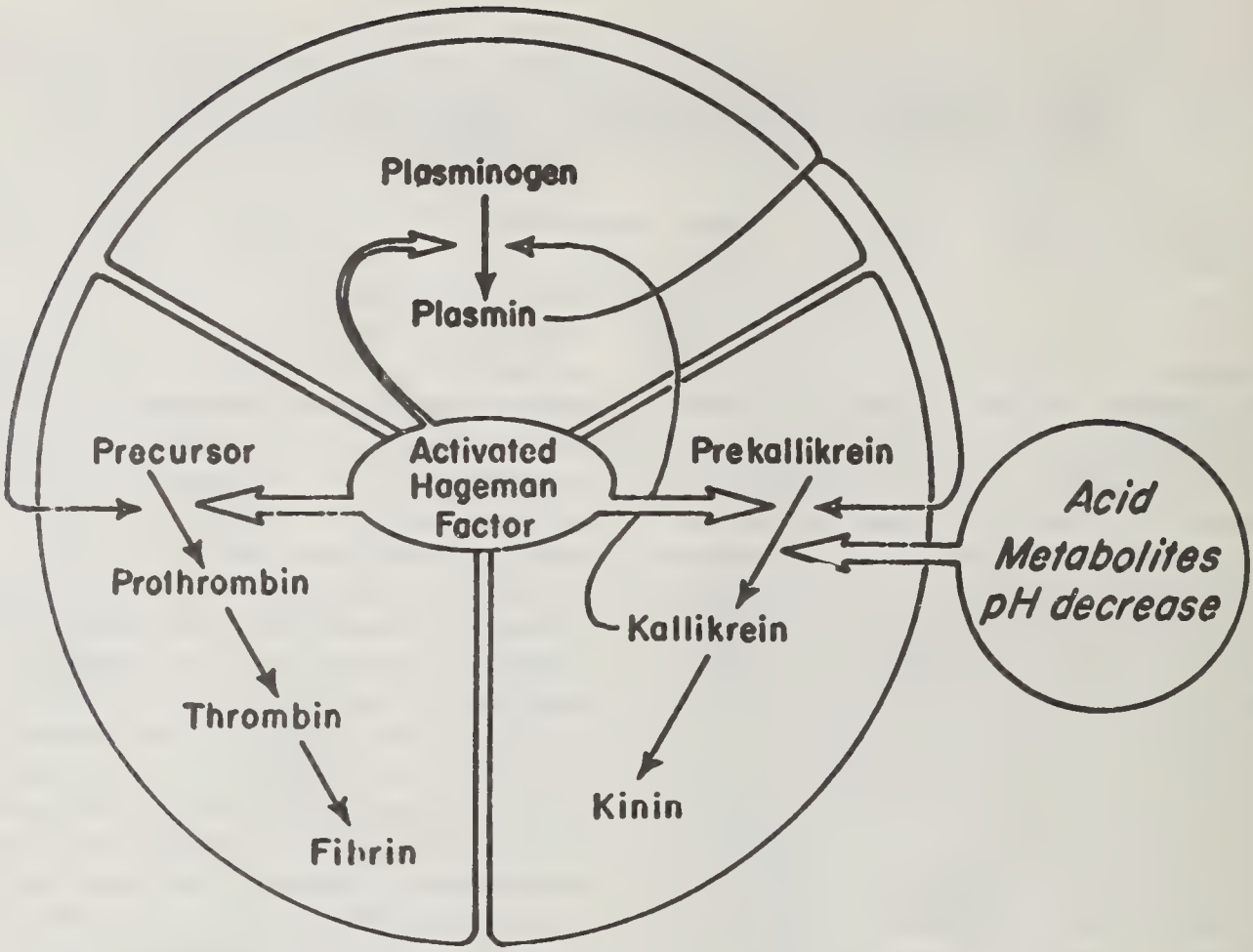


FIGURE 1.—Interrelationship amongst the blood coagulation, fibrinolysin and vasoactive polypeptide systems. The role of acid pH in activation of the systems.

tion. A left side thoracotomy and pericardotomy was performed. A single ligature was placed loosely around the origin of the anterior descending branch of the left coronary artery. The ends of the ligature were exteriorized through the chest wall and the pericardial sac and chest wall closed (Fig. 3). After a 7-day recovery period, the animals were prepared for recording of ECG and arterial blood pressure via a cannula inserted into the femoral artery (under local anesthesia). Control venous blood samples were drawn and base line physiologic parameters were obtained. Seven dogs received saline, 10 mls/kg, while the other 7 received a proteinase inhibitor, TRASYLOL, 50,000 units/kg intravenously just prior to occlusion. The coronary ligatures then were tightened suddenly and blood samples drawn at rapid intervals thereafter. The acute phase was followed for 30 minutes and then from 24 hours to 8 days.

Autopsies were carried out on all animals as well as histopathology, but will not be reported in this preliminary communication.

Biochemical-Hematological Studies

Biochemical and hematologic studies were carried out in accordance with previously published methods.[9] The following parameters were measured: hematocrit, Lee White clotting time, prothrombin time, plasma fibrinogen, euglobulin clot lysis time, kininogen, kinin, kininase, and SGOT.

RESULTS

Two of the control dogs died acutely after the occlusion and one died 24 hours later of heart rupture. One TRASYLOL-treated dog died acutely while one died after 24 hours. All dogs

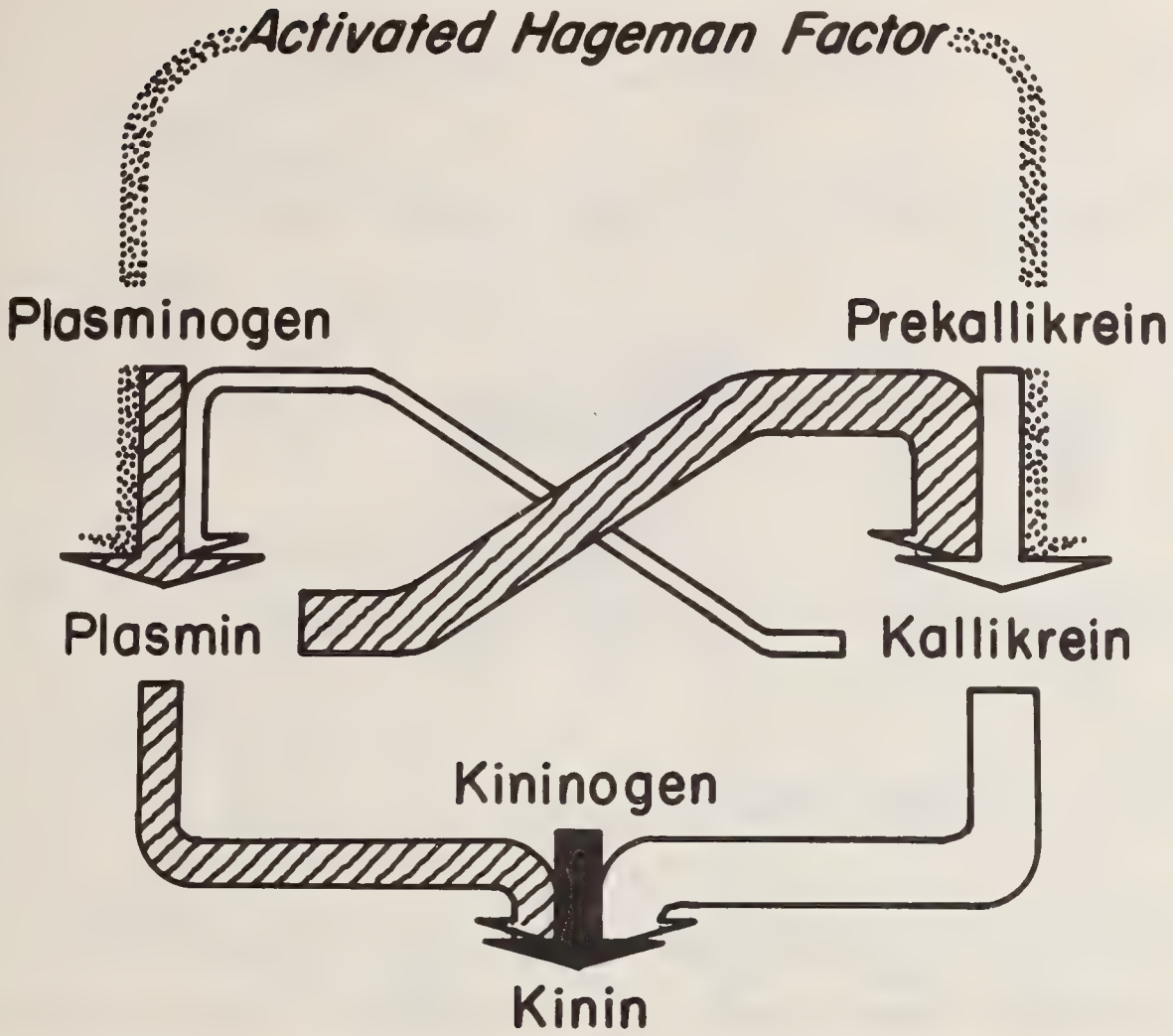


FIGURE 2.—Interrelationship between the fibrinolysin and vasoactive polypeptide syste highlighting ability of both plasmin and kallibrein to form kinin directly and indirectly.

showed varying degrees of cardiac arrhythmias and other ECG changes. The saline control dogs evidenced more pain at the moment of occlusion as indicated by extensive body movement, discomfort, and vocalization.

Figs. 4 and 5 summarize the biochemical data. All percent changes are based on the control base line values after saline or TRASYLOL. While standard errors are not indicated, the levels of significance (at the .05 level) between the control and TRASYLOL-treated groups are indicated by a black dot. Hemoconcentration did not occur until 24 hours after occulsion, and then only in the control dogs. Hematocrit (HCT) differences between the control and drug-treated groups were significant only at 24 hours and 8 days. Lee White clotting times (LWCT) increased significantly in the control dogs during the acute phase and at 5–6 days after occlusion. Prothrombin (PROTH) levels were decreased throughout the experimental period. Fibrinogen (FBGN) levels increased throughout the experimental period in the control dogs. This increase was not as prominent in the drug-treated dogs, differing significantly from the control dog levels during the acute phase. An increase in euglobulin clot lysis activities (shortened euglobulin clot lysis times—ECLT) was noted for both groups at 30 minutes post-occlusion, and remained so for the TRASYLOL-treated dogs; while the activity decreased in the saline control dogs. There were kininogen (KNGN) decreases in the TRASYLOL-treated dogs, which, while not of large magnitude initially, did differ significantly from the control dogs. Larger decreases did occur later in the more chronic phase. No kinin activity was detected in either of the two groups

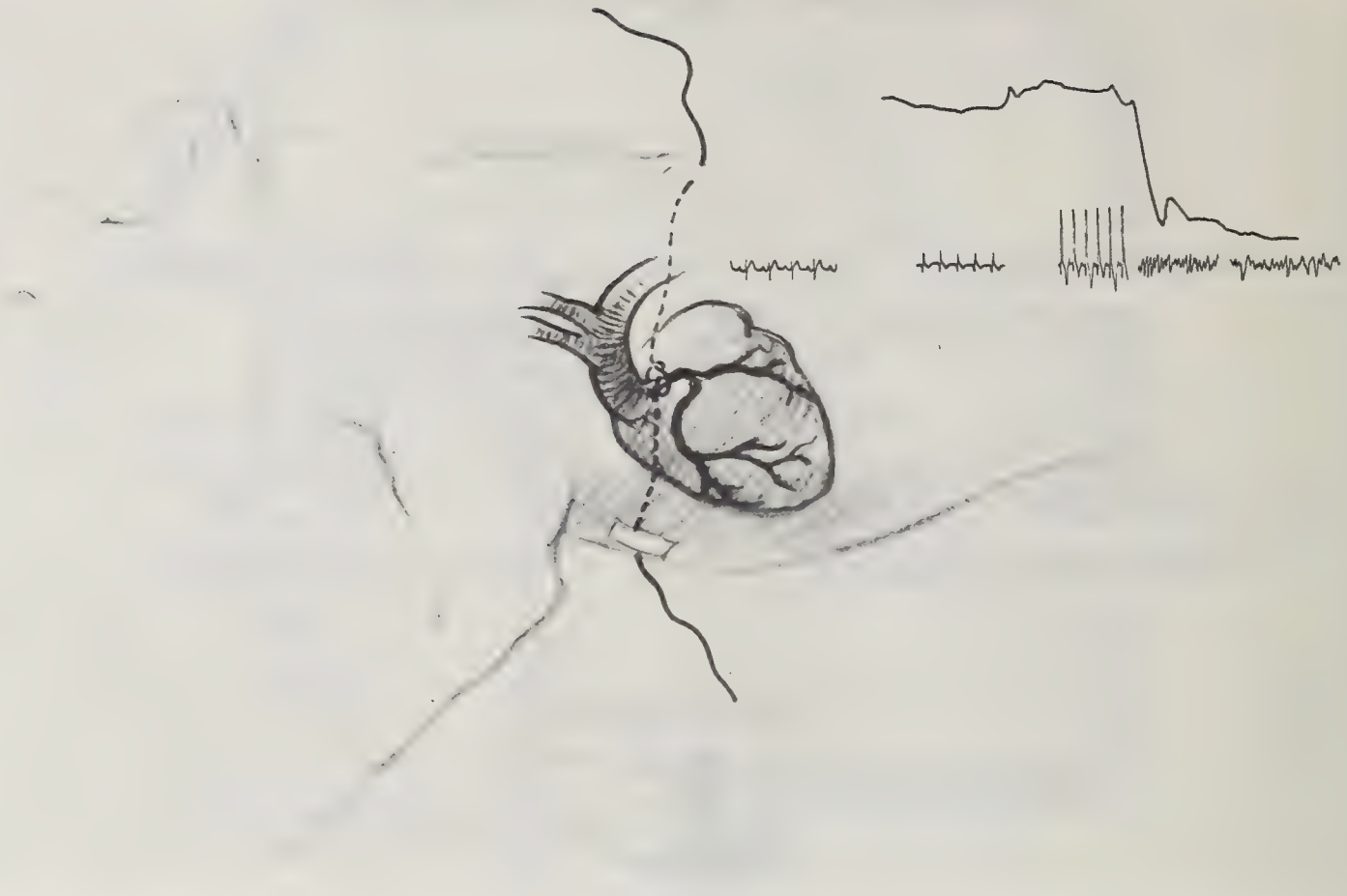


FIGURE 3.—Representation of the surgical procedure for producing acute coronary occlusion in the unanesthetized dog.

at any of the time periods. Kininase (KNASE) activity was increased greatly at all time periods in the control dogs, while this activity remained at near-normal levels in the drug-treated dogs. SGOT values were increased in both groups of dogs at the 24-hour time period.

SUMMARY

These studies are but preliminary and, as such, do not permit interpretation at this time. An experimental model has been described that allows for the sequential analysis of components of three protease systems prior to and during an acute myocardial ischemic episode. It would appear that more frequent blood sampling would be valuable in determining the direction of change during the initial acute phase of the occlusion. In addition, biochemical changes as determined from venous samples may or may not reflect critical changes occurring at the local myocardial site. Blood samples drawn directly from the coronary vessels at the time of occulsion might allow for more accurate evaluation of those immediate changes occurring at the local site.

Additional studies are planned to determine the significance of some of the biochemical changes, to be correlated with histopathology data. The effect of protease inhibitor treatment also will be assessed, both in an attempt to clarify the role of proteases in this condition, and as a therapeutic possibility. While thrombolytic agents are considered to form one of the bases for rational therapy throughout most stages following coronary occlusion [10], inhibitor therapy may be worthy of consideration in the very early post-occlusive phase, where protease activation may be dominant but undetected hithertofore.

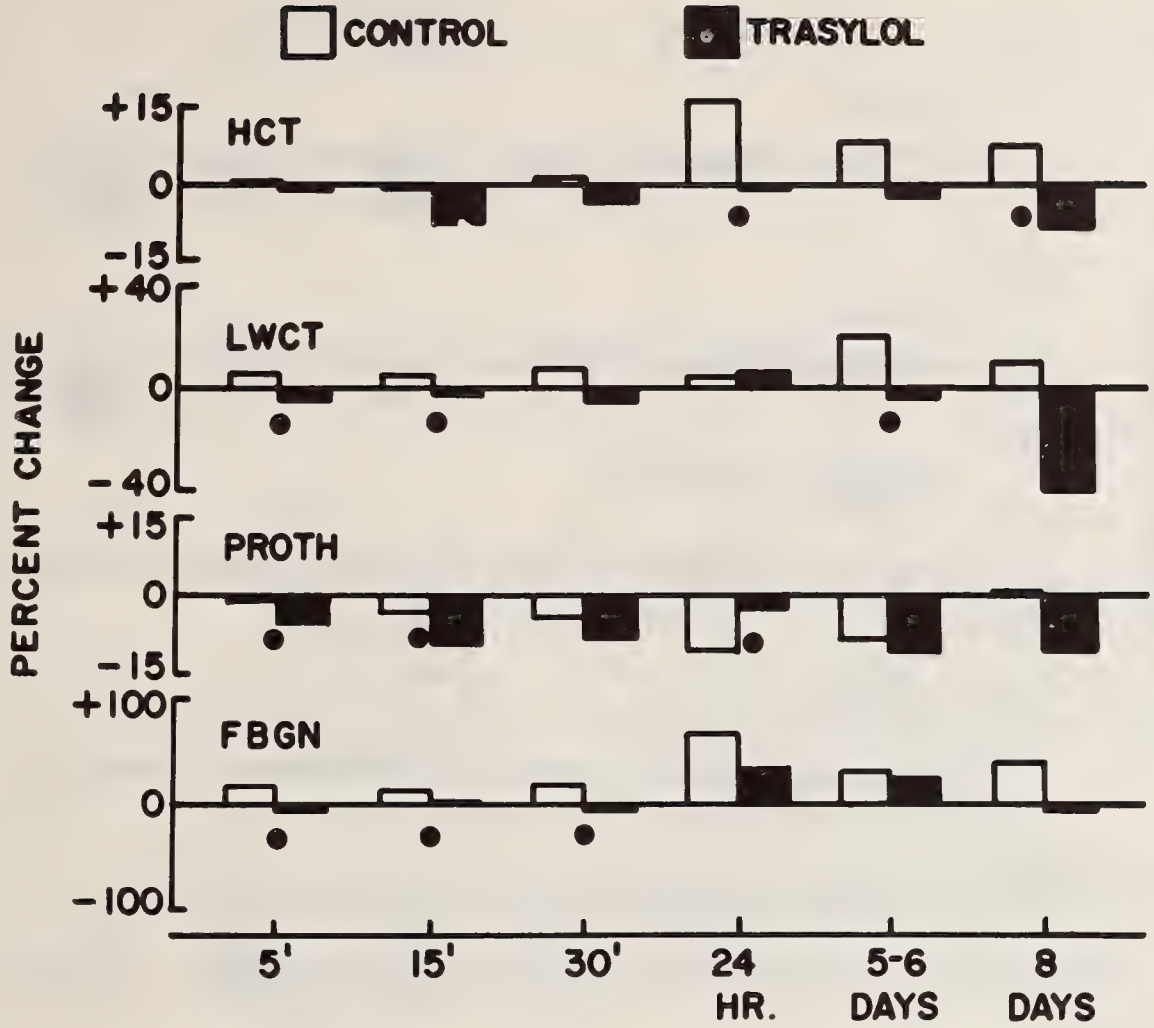


FIGURE 4.—Percent change from baseline values of hematocrit (HCT), Lee White clotting time (LWCT), prothrombin (PROTH.), and fibrinogen (FBGN) following acute coronary occlusion. TRASYLOL animals received 50,000 units/kg intravenously just prior to occlusion.

REFERENCES

1. EDWARDS, J. E. What is myocardial infarction? Circulation Suppl. to Vols. 39 and 40. IV–5, 1969.
2. BRACHFELD, N., Maintenance of cell viability. Circulation Suppl. to vols. 39 and 40. IV–202, 1969.
3. WEISSLER, A. M., KRUGER, F. A., BABA, N., SCARPELL, D. G., LEIGHTON, R. F., and GALLIMORE, J. K. Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium. J. Clin. Invest. 47:403, 1968.
4. WILKENS, H., BACK, N., STEGER, R., and KARN, J. The influence of blood pH on peripheral vascular tone: Possible role of proteases and vaso-active polypeptides. In *Shock: Biochemical Pharmacological, and Clinical Aspects*. Eds. A. Bertelli, and N. Back. Plenum Press, New York, p. 201, 1970.
5. BACK, N. The fibrinolysis system and vasoactive kinins. Fed. Proc. (Symposium) 25:77, 1966.
6. SICUTERI, F., and DEL BIANCO, P. L. Vasoattivita dell endecapeptide fisalemina nell'uomo. Settini. Med. 52:1221, 1964.
7. SICUTERI, F., DEL BIANCO, P. L. and ANSELMI, B. La bradichinina (sistema chininogen-chinine) nella patogenesi del dolore e dello shock miocardica. Boll. Soc. Ital. Cardiol. 11:1362, 1966.
8. BASSANGE, E., KUCHARCZYK, M., VON RESTORFF, W., and WERLE, E. Effect of bradykinin potentiating peptide on coronary circulation in conscious dogs. In *Vasopeptides: Biochemistry and Pathophysiology*. Eds. N. Back and F. Sicuteri. Plenum Press, 1972. In Press.
9. BACK, N., WILKENS, H., and STEGER, R. Proteinases and proteinase inhibitors in experimental shock states. Ann. N.Y. Acad. Sci. 146:491, 1968.
10. RUEGSEGGER, P. Fibrinolytic therapy of myocardial infarction: Theory and clinical results. Angiologica 5:136, 1968.

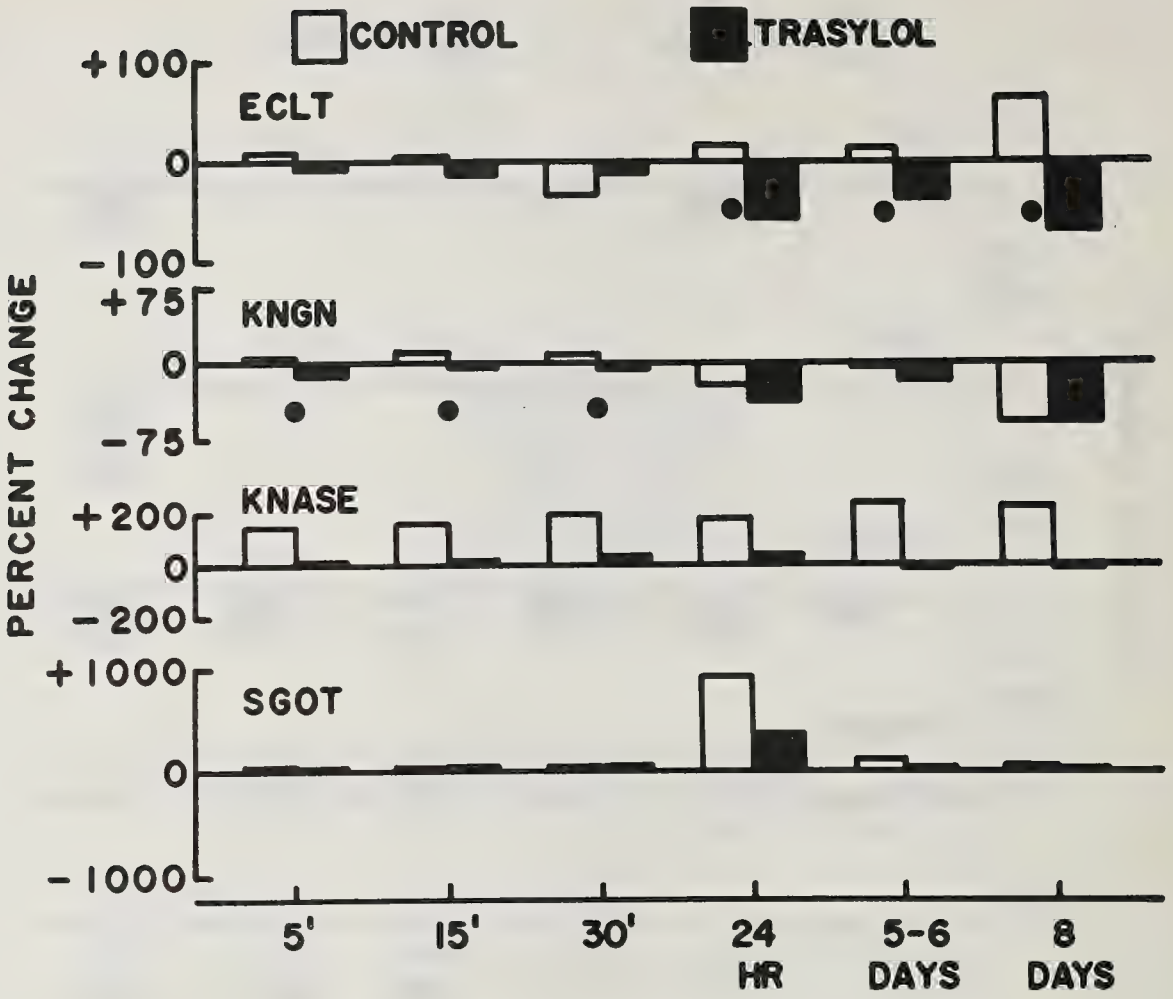


FIGURE 5.—Percent change from base line values of euglobulin clot lysis time (ECLT), kininogen (KNGN), kininase (KNASE), and serum glutamicoxalacetic transaminase (SGOT) following acute coronary occlusion. TRASYLOL animals received 50,000 units/kg intravenously just prior to occlusion.

CHAPTER III

PULMONARY MODELS

Pulmonary Hypertension in Young Macaca Arctoides Monkeys: A Sequela to Monocrotaline Intoxication

Hypoxic-Induced Bovine Pulmonary Hypertension

A Model for Closed Thoracic Observation of the Pulmonary Microcirculation in Shock

A Pulmonary Xenograft Model for Microcirculatory Observation

Acute Bovine Pulmonary Emphysema: Evaluation of Tryptophan Induction

Chronic Obstructive Lung Disease in Horses

Pathophysiologic Assessment of a Canine Model of Induced Acute Respiratory Insufficiency

Medial Hyperplasia of the Pulmonary Arteries of Cats

Alveolar, Bronchiolar and Pulmonary Vascular Responses of Isolated Rat and Guinea Pig Lungs

The Effect of Hypoxia on the Pulmonary Microcirculation

Cinemicroscopy of the Pulmonary Microcirculation in Shock

Models of Cardiopulmonary Function in Calves

PULMONARY HYPERTENSION IN YOUNG MACACA ARCTOIDES MONKEYS: A SEQUELA TO MONOCROTALINE INTOXICATION

J. R. Allen, J. M. Jenny and C. F. Chesney*

Infant *Macaca arctoides* monkeys (stumptail) were given injections of the plant alkaloid monocrotaline ($C_{16}H_{20}O_6N$) on four occasions during a six month period. Within the terminal month or two of the experiment, there was an increase in hemoglobin, hematocrit, red cell volume and arterial pCO_2 and a decrease in arterial pO_2 and pH. Increases in right heart and pulmonary artery pressures were also indicative of changes in the pulmonary vasculature. Prior to death, the least exertion by these animals produced marked cyanosis. Grossly, the major changes noticed at necropsy were related to the heart and lungs. There was hypertrophy of the myocardium and a decided right heart dilatation. The lungs were uniformly firm and failed to collapse readily when exposed to atmospheric pressure. Microscopically, there were extensive modifications in the pulmonary vasculature. Platelet and fibrin thrombi occluded numerous interalveolar capillaries. The walls of many arterioles and small muscular arteries were hyalinized while in others there was hypertrophy of the smooth muscle and endothelium, both of which produced partial or complete occlusion of the lumens. Fractured endothelial linings of the larger pulmonary arteries predisposed to the accumulation of blood components throughout the wall and to the development of fibrin thrombi within the lumen of the affected vessels. The myocardium of the right heart was edematous, the endocardium fibrotic and the arterial vessel walls disrupted.

INTRODUCTION**

It has been established that subhuman primates offer the most ideal experimental model to evaluate two maladies of man, Budd-Chiari syndrome and veno-occlusive disease. Within a few months following the administration of the pyrrolizidine alkaloid, monocrotaline ($C_{16}H_{26}O_6N$) to adult *M. arctoides* monkeys, segments of the hepatic venous system were obstructed.[1-3] The animals developed ascites, portal hypertension, varices, hepatic fibrosis and peliosis hepatis. However, cardiorespiratory lesions in these animals were not apparent. The present communication is concerned with modifications in the above experimental procedure whereby similar doses of monocrotaline produce sufficient alteration in the pulmonary vascular bed of monkeys to facilitate interalveolar fibrosis, pulmonary hypertension, cardiac hypertrophy, and a right heart dilatation (cor pulmonale). It is suggested that monocrotaline intoxicated monkeys having cardiorespiratory lesions offer a readily produced experimental model to study human respiratory and cardiac maladies related to hypertension.

MATERIALS AND METHODS

Twelve *Macaca arctoides* monkeys, 30 days of age, weighing approximately 650 grams, and of both sexes, were employed in this study. Following a complete blood study which included hemoglobins (Hb), hematocrits (Hct), white blood cell counts (WBC), differential counts, serum bilirubins, serum glutamic oxalacetic transaminase (SGOT), total serum protein (TP), and electrophoretic patterns of the serum proteins, the animals were subcutaneously injected initially with 30 mg of monocrotaline per kg of body weight. They were subsequently given 60 mg of monocrotaline per kg of body weight on the second, fourth, and sixth month of the experiment. When the animals developed respiratory symptoms and displayed a relative increase in circulating erythrocytes as determined by blood Hcts and an absolute increase as determined by red cell volume,[4] the arterial and venous blood was analyzed for its hydrogen ion concentration, pCO_2 and pO_2,*** and right heart and pulmonary artery pressures were determined via cardiac catheterization.

* Department of Pathology and Wisconsin Regional Primate Research Center, University of Wisconsin Madison, Wisconsin

** Supported in part by U.S. Public Health Service Grants HE10941, RR00167 and 1F02-ES46357

*** Radiometer (Astrup method) Blood Gas Analyzer, type AME1, The London Co., Westlake, Ohio.

When it became clinically obvious that the animals were in a terminal state, they were sacrificed. The thoracic cavity was opened immediately and small segments of the heart, lung, and liver were taken for electron microscopy. Sections were taken of all major organs for light microscopic evaluations. Preparation of the tissue for light microscopic and ultrastructural studies were similar to methods previously described.[2]

RESULTS

The average survival time of the infant monkeys was 241 days (199–325). Throughout the early stages of the experiment the animals showed few outward effects of monocrotaline intoxication. There were only slight increases in SGOT and serum bilirubin, while A/G ratios of the serum and the total serum protein were not altered appreciably. Within a month or two prior to death, there was an increase in Hct from 40% to 60% and a concomitant rise in hemoglobin concentration (12 vs. 17 g %). Five animals having Hcts in excess of 55% were injected with red blood cells tagged with Cr^{51}. There was a 20% increase in the circulating red cell volume when compared to that of normal animals of similar age. At the time when the monkeys had a decrease in arterial and venous pO_2 (110 vs. 42, and 55 vs. 27 mm Hg, respectively) a concomitant increase in arterial pCO_2 (23 vs. 42 mm. Hg) was recorded. The pH of the arterial blood was also decreased in the experimental animals during this period (7.49 vs. 7.27). Right ventricular pressures taken on these animals exceeded 50 mm Hg as opposed to an average of 20 mm Hg in the control animals. The pulmonary artery pressures in the control monkeys averaged 16 mm Hg, while those of the monocrotaline group had values ranging between 35 and 45 mm Hg. As the experiment progressed, respiratory insufficiency became more severe. Prior to death, the least exertion produced marked cyanosis, and frequently required oxygen therapy to facilitate survival.

In order to obtain ideal tissues for ultrastructural studies, the animals were sacrificed when cardiorespiratory dysfunctions reached a stage that death was imminent. At the time of necropsy, the bodies of all the experimental animals were well nourished. The major pathologic alterations were located in the heart and the lungs; although pink in color the lungs were quite firm and failed to collapse readily on exposure to atmospheric pressure. There was also hypertrophy of the left heart and marked dilatation and hypertrophy of the right heart. The right ventricle extended to the apex of the heart and the walls were decidedly thickened. The dilatation of the right heart was also exemplified by the increase in size of the bicuspid valve which averaged 4.5 cm in experimental animals and 2.0 cm in the control animals.

Microscopically, there were extensive changes in the respiratory tissue. Partial to complete occlusion of the capillaries, arterioles, and small muscular arteries throughout the lungs was observed (Figure 1). In addition, fibrous thickening of the alveolar septum was prominent in the areas closely associated with the occluded vessels. Thrombosis and arteritis involving the larger arteries were present in five experimental animals.

There was fragmentation, desquamation, and dehiscence of the capillary endothelium. These affected capillaries invariably were filled with platelet and fibrin thrombi. The changes in the pulmonary arteries were related to the mor-

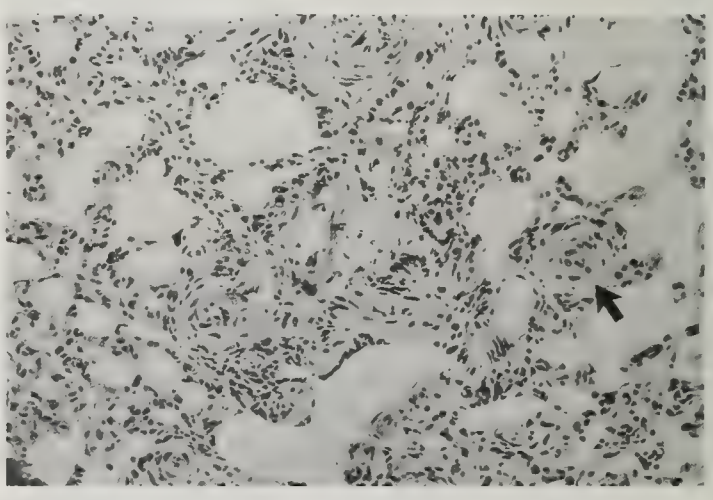


FIGURE 1.—Lung section from a monocrotaline intoxicated animal that survived six and one half months. There is a decided thickening of the arterial walls primarily due to muscle hypertrophy (arrow). Interalveolar septa are also increased in thickness as a result of increased fibrous connective tissue, fluid and cellular components. H & E stain; x 176.

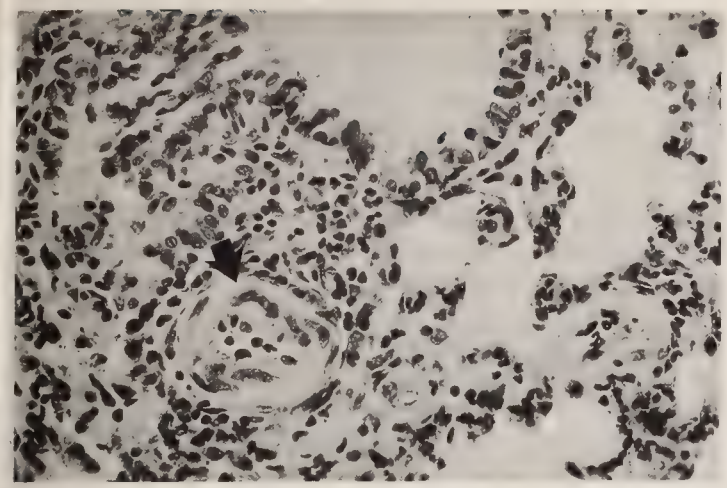


FIGURE 2.—Hypertrophy of smooth muscle cells in the wall of a small muscular artery is depicted (arrow). Loss of the muscular components and replacement by hyaline material is shown in closely associated arteriole and muscular artery. H & E stain; x 413.

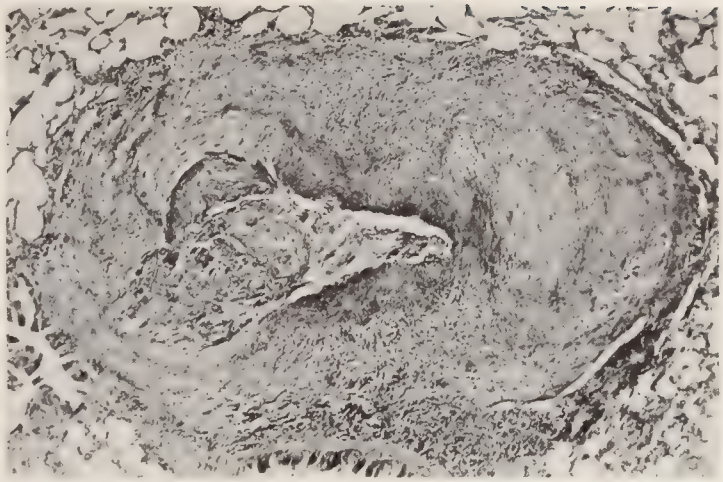


FIGURE 4.—Thrombi developed in the lumens of large pulmonary arteries of 5 animals. Note the predominantly fibrinous nature of the thrombus. The entire vessel wall is edematous and numerous leukocytes are obvious throughout. H & E stain; x 45.

phological alterations in the vessel walls. In many of the small muscular arteries, occlusion was attributed to the extensive hypertrophy of the endothelial and smooth muscle cells (Figure 2). These cellular modifications resulted in reduction or obliteration of the lumens of the affected arteries. Other arteries had obvious rents in their endothelium. Blood cells and plasma accumulated beneath these injured areas leading to further disruption of the vessel wall. There were also other arteries whose walls and lumen were partially or completely replaced by hyaline material (Figure 2). Electron microscopically, these vessel walls were composed primarily of amorphous and fine fibrillar material similar to basal lamina.

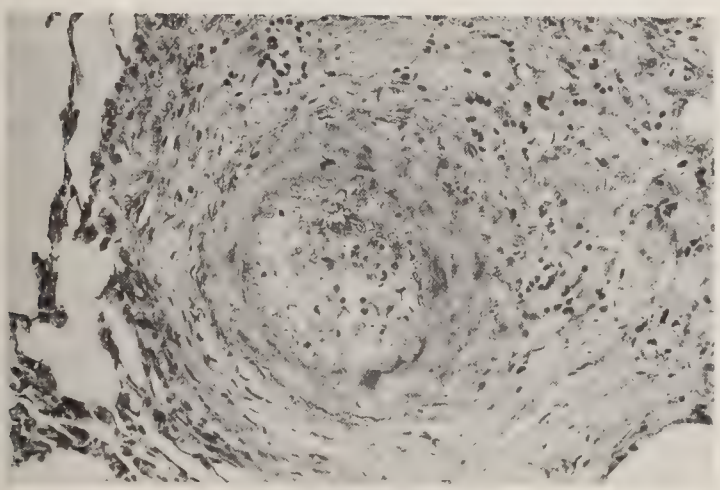


FIGURE 3.—Extensive intimal disruption is depicted in this medium sized pulmonary artery. Notice the distance between the internal elastic lamina and remains of endothelium. Edematous disruption is also apparent in the medial portion of the wall. Mononuclear cells are prevalent between the fibrous strands of the adventitia. H & E stain; x 158.

In the larger arteries there were breaks in the endothelial lining and intimal disruption produced by the extravasated blood (Figure 3). In the more severely affected vessels, erythrocytes, leukocytes, and plasma were also present between the muscular components of the media and adventitia. In many instances, fibrin and platelet thrombi developed in areas of the vessel having a disrupted endothelial surface (Figure 4).

In close association with the disrupted capillaries, arterioles, and arteries were thickened alveolar walls consisting of fibrous connective tissue, macrophages, and histiocytes. In well demarcated areas of the lungs of 4 animals, there was epithelialization of the alveolar lining cells. These closely associated large columnar cells contained centrally located nuclei and numerous cytoplasmic organelles.

The myocardium of the left ventricle had enlarged muscle cells, but appeared normal otherwise. The edematous right ventricular musculature of all the infants was characterized by widely separated myofibrils, numerous areas of organelle-free matrix and numerous variable-

sized membrane enclosed droplets. The thickened endocardium of the right ventricle was attributed to edema, focal hemorrhage and a decided subendothelial proliferation of fibrous connective tissue.

The arteries throughout the right ventricle were also affected. Their entire walls were edematous, components were widely separated, and extravasated blood cells were prevalent throughout the wall. Fractures in the endothelial lining were apparent in some of these vessels.

The hyperplastic bone marrow of the infant monkeys displayed numerous megakaryocytes and erythroid and myeloid elements. The general morphological features of the livers were fairly well maintained with the exception of isolated areas of vascular occlusion involving the small hepatic veins. The development of extensive hepatic scarring and the formation of endothelial lined sinuses so prevalent in older monocrotaline intoxicated monkeys[1-3] was inapparent in the livers of the infant animals.

DISCUSSION

Infant *Macaca arctoides* monkeys develop extensive pulmonary vascular lesions following the administration of the pyrrolizidine alkaloid, monocrotaline. Pulmonary hypertension, cardiac hypertrophy, and right heart dilatation are aftermaths of the vascular changes. When adult monkeys are given similar doses of monocrotaline, only minor changes are noticed in the lungs; however, extensive liver lesions develop.[1-3] This variation in response is attributed to the means by which the alkaloid is metabolized by the hepatic microsomal enzymes of the two age groups.[5] It has been demonstrated that the microsomes from infant livers do not have the ability to convert monocrotaline into pyrroles (the liver toxin) as readily as microsomes from adolescent or adult livers. It has been postulated that metabolites other than the pyrroles may give rise to the lung lesions,[6] and the enzymes responsible for these metabolic alterations are more active in the microsomes of the younger animals. Regardless of the exact mechanism associated with the age dependent response, infant monkeys must be employed if acute pulmonary vascular lesions are desired.

Cor pulmonale, liver necrosis, and hepatic and pulmonary vascular lesions, similar to those seen in primates, have been reported in rodents given monocrotaline.[7,8] The major difficulty in employing these animals as experimental models to study pulmonary or hepatic vascular lesions is related to the inability of the investigator to predict which of the two organs will be affected most severely under a given set of circumstances. Rats of similar breeding, weight, and sex may develop pulmonary lesions when injected on one day and hepatic lesions when litter mates are injected on the following day. It is also difficult to predict the survival rates of rats with acute pulmonary lesions. Pulmonary edema and hemorrhage likely related to altered vascular permeability, invariably present complications during the initial phases of monocrotaline intoxication and are largely responsible for heavy mortality. Such complications do not arise when subhuman primates are employed as experimental animals. By varying the age of the monkey, the specific lesions produced by monocrotaline can be accurately predicted and promptly developed in the absence of mortalities.

SUMMARY

Monocrotaline intoxicated monkeys offer a readily produced experimental model to study pulmonary hypertension and right heart failure in an animal having similar tissue responses and closely related phylogenetically to man.

ACKNOWLEDGMENTS

The authors express appreciation to Dr. J. A. Will for his assistance in the determinations of pulmonary artery and right heart pressures.

REFERENCES

1. Allen, J. R., Carstens, L. A., and Olson, B. E. Veno-occlusive disease in *Macaca speciosa* monkeys. Amer. J. Path. 50:653, 1967.
2. Allen, J. R., Carstens, L. A., and Katagiri, G. J. Sequential ultrastructural changes in hepatic veins of monkeys with veno-occlusive disease. Arch. Path. 87:279, 1969.

3. ALLEN, J. R., and CARSTENS, L. A. Monocrotaline induced Budd-Chiari syndrome in monkeys. Amer. J. Digest Dis. 16:111, 1971.
4. STERLING, K. and GRAY, S. J. Determination of the circulating red cell volume in man by radioactive chromium. J. Clin. Invest. 29:1614, 1950.
5. ALLEN, J. R., and CHESNEY, C. F. Effect of age on development of cor pulmonale in non-human primates following pyrrolizidline alkaloid intoxication. Exper. Molec. Path. (In press).
6. ROGERS, A. E., and NEWBERNE, P. M. Lasiocarpine: Factors influencing its toxicity and effects on liver cell division. J. Toxicol. Appl. Pharma. 18:356, 1971.
7. KAY, J. M., and HEATH, D. *Crotalaria spectabilis. The Pulmonary Hypertension Plant.* Charles C. Thomas, Springfield, 1969.
8. HAYASHI, Y., and LALICH, J. J. Renal and pulmonary alterations induced in rats by a single injection of monocrotaline. Proc. Soc. Exp. Biol. Med. 124:392, 1967.

HYPOXIC-INDUCED BOVINE PULMONARY HYPERTENSION

A. F. Alexander, C. S. Card, R. S. Jaenke, J. L. Hicks and D. H. Will*

Hypoxic induced pulmonary hypertension has been indirectly recognized for years in the bovine species through the existence of a syndrome known as High Mountain or Brisket Disease, characterized by the development of pulmonary hypertension and resultant congestive heart failure. To study the possibility of heritable factors in resistance and susceptibility to hypoxic induced pulmonary hypertension, two groups of cattle were established based on the degree of pulmonary hypertension occurring at high altitude and on the presence or absence of high mountain disease in their history. Offspring of the susceptible parentage have shown a consistently greater pulmonary hypertension and vascular resistance to both acute and chronic hypoxia than have the progeny of the resistant group. The reasons for the differences in response remain unclear, although some evidence implicates a greater degree of airway hypoxia in the more susceptible animals. Ultrastructural studies of hypertensive pulmonary arterioles suggest changes in the metabolic activity of medial smooth muscle cells.

INTRODUCTION**

Pulmonary hypertension occurs in man and animals in association with many diseases and conditions. One etiologic factor of pulmonary hypertension is inhalation hypoxia. In the past decade hypoxic induced pulmonary hypertension has become a well known entity in cattle. It had been indirectly recognized in that species for years by the existence of a clinical syndrome called High Mountain or Brisket Disease.[1] When exposed to chronic or long term hypoxia cattle distinguish themselves from most other species by developing more severe levels of pulmonary hypertension. Bovine high mountain disease, characterized by the development of severe pulmonary hypertension and resultant congestive right heart failure,[2] has been recognized in the mountainous areas of Colorado since the end of the 19th century. The first report pertaining to the disease was by Glover and Newsom in 1915.[3] The spontaneous disease is reported from the Rocky Mountains of North America, the Andes Mountains of South America, and the mountainous elevations of East Africa.

The syndrome develops most frequently in animals resident in altitudes above approximately 7,000 feet. However, uncomplicated cases have been recognized at elevations of approximately 5,000 feet, and the pulmonary hypertensive effect of mild chronic hypoxia in calves has been confirmed.[4] The overall incidence of the disease is estimated to be between 0.5 to 2% in native cattle residing above 7,000 feet, with a direct relationship existing between resident altitude and incidence. All ages and both sexes are affected. In Colorado and Wyoming, a higher incidence is seen in animals under one year of age, and in animals newly imported to the high altitudes from low elevations. This epidemiological pattern is also observed in the Andes and East Africa. In contrast, at altitudes of approximately 5,000 feet in Montana, the incidence is noted to be higher in adult cattle. In Colorado, there is a definite seasonal variation in the incidence of the natural disease in native cattle, with the occurrence being highest in the fall and winter, especially following periods of inclement weather.

Clinical signs in an affected animal are those of congestive heart failure and include subcutaneous edema, especially in the ventral, pectoral and cervical areas, distention and pulsation of the jugular vein, ascites, and frequently a profuse fluid diarrhea. The animals tend to show labored breathing, and upon forced exertion may collapse and die. The removal of affected animals to lower elevation results in sponta-

* Departments of Pathology and Physiology-Biophysics College of Veterinary Medicine and Biomedical Sciences Colorado State University, Fort Collins, Colorado 80521.

** Supported by Grant HE 08759 from the National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland.

neous clinical recovery in approximately 50% of the cases.

Pathologically, affected animals have lesions of generalized passive congestion with *edema, hydrothorax, ascites, and cor pulmonale.* Post mortem pulmonary arteriography has demonstrated dearborization of the pulmonary arterial tree,[5] indicative of an increased precapillary resistance to flow. Light microscopic examination of the lungs has revealed medial smooth muscle hypertrophy and adventitial proliferation around the small pulmonary arteries and arterioles.[6,7] Distal occlusion of small arteries by thrombi, emoli, or intimal proliferation is not present, nor has morphological evidence of postcapillary obstruction been recognized.

METHODS

Experimental studies have demonstrated that normal cattle taken from a low to a high altitude develop significant pulmonary hypertension.[8,9] Increasing the test altitude hastens the development of pulmonary hypertension, indicative of the apparent marked sensitivity of the bovine pulmonary vasculature to changes in oxygen tension.[9] The effect of simulated altitudes in a hypobaric chamber of 15,000 and 20,000 feet on eight calves is shown in Figure 1. The time course for the development of severe pulmonary hypertension and congestive failure was shorter for the animals at 20,000 feet. Likewise, the time required to develop severe pulmonary hypertension at 15,000 feet was slightly less than at 12,700 feet,[9] which in turn was less than at 10,000 feet.[8] In general, in the absence of significant increases in flow, blood viscosity, and pulmonary arterial wedge pressures, the pulmonary hypertension has been attributed to a decrease in the cross sectional area of the pulmonary vascular bed at the precapillary level.

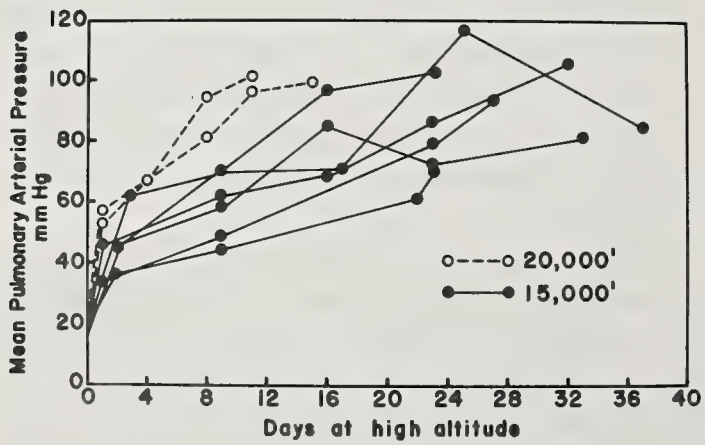


FIGURE 1.—Effect in cattle of 15,000 and 20,000 feet altitude (hypobaric chamber) on pulmonary vascular pressure.

The magnitude of the pulmonary hypertension, which develops when cattle are moved to high altitudes shows considerable variation. Some animals develop only moderate pulmonary hypertension, while others develop severe hypertension leading to congestive right heart failure.[8,9] None of the functional or morphological measurements have served to clearly explain the marked individual differences in the response of cattle to high altitude.

In a study of cattle native to and resident at 5,000, 8,500, and 10,000 feet altitude,[10] only low to moderate pulmonary hypertension was observed in the animals at the higher altitude (Table I). It is probable that both natural and artificial selection have occurred at high altitude to produce a native cattle population with a more hyporeactive pulmonary vascular bed and a low incidence of high mountain disease. This selection process may have tended to eliminate the hyperreactive type of animal.

Many unpublished statements, implications, and observations have been made that strongly indicate that the susceptibility of cattle to high mountain disease may be partially but significantly heritable. Although difficult to prove on an epidemiologic study basis, certain breeds appear to have greater susceptibility to the spon-

TABLE I.—*Mean Pulmonary Arterial Pressures in Cattle Resident at Different Altitudes*

Age		Resident Altitude in Feet		
		5,000	8,500	10,000
4–8 years	Number	4	15	17
	Mean PAP*	29.8	38.2	37.4
	Std. Dev.	2.5	6.4	7.9
2 years	Number	10	16	30
	Mean PAP	30.1	28.5	27.7
	Std. Dev.	4.3	4.6	9.8
4–6 months	Number	10	15	17
	Mean PAP	30.1	28.5	37.7
	Std. Dev.	2.3	4.8	16.8

* Pulmonary arterial pressure, mm Hg.

TABLE II.—*Three-Year Herd History Implicating a Specific Male in the Incidence of High Mountain Disease*

Total number calves produced	359
Number developing HMD	8
Total number sired by suspect bull	39
Number of offspring developing HMD	5

Suspect sire also developed HMD.

taneous disease. Likewise, in certain herds, a high incidence factor has been traced to offspring of particular dam or sire combinations (Table II). In contrast, the disease has not been observed in the native Criollo cattle in the Peruvian Andes where a high incidence occurs in imported cattle.

For these reasons, studies of heritable susceptibility were undertaken. The objective was to establish two distinct groups of cattle, one classified as "susceptible" and the other as "resistant" to the hypoxic stress of high altitude. The establishment of the parent susceptible and resistant herds was accomplished by the selection of two groups of cattle based on the presence or absence of high mountain disease in their history, and their level of high altitude induced pulmonary hypertension. Susceptible parent stock females were selected from heifers between 6 and 18 months of age, not necessarily native to high altitude, but which developed uncomplicated high mountain disease at altitudes of 8,000 feet or higher. Similar criteria were used in the selection of a suitable sire for this group. Females for the resistant parent herd were selected from heifers of at least one year of age who were born, raised and resided at altitudes of 9,000 feet or above and at those altitudes had low pulmonary arterial pressures. To identify these animals catheterization studies were performed on 49 heifers meeting the age, residence and altitude requirements. The 15 heifers with the lowest mean pulmonary arterial pressures were selected.

Similar criteria were used in choosing a male to serve as sire for the resistant group. Only three males could be found which met the requirements of being born and raised at altitudes of greater than 9,000 feet. The animal with the lowest mean pulmonary arterial pressure was chosen as group sire. The parent susceptible herd, therefore, consisted of animals which had developed and recovered from clinical high mountain disease while the resistant herd consisted of animals native to and residing at altitudes greater than 9,000 feet but which had low pulmonary arterial pressures and no clinical history of high mountain disease (Figure 2).

To produce the first year progeny, ten of the susceptible parent females were bred to a susceptible male. Eight viable calves were produced, four females and four males. Fifteen resistant parent famales were bred to the resistant male. Eleven viable calves were produced, four females and seven males (Figure 2). Cardiorespiratory and hematological studies were carried out on each calf at 10, 30, 60, and 90 days of age at an altitude of 5,000 feet (Fort Collins, Colorado) and at 10 and 90 days after two hours exposure in the hypobaric chamber at a simulated altitude of 15,000 feet.[11] When the minimum age within the two groups of calves was three months, they were moved to a mountainous altitude of 10,000 feet for chronic hypoxic exposure. During the ensuing six month period, periodic cardiorespiratory and hematologic studies were made.

In the susceptible group of calves, higher levels of mean pulmonary arterial pressure were observed at 5,000 feet at 10, 30, 60, and 90 days and at 15,000 feet at both 10 and 90 days of age (Figure 3). The increase in pulmonary arterial pressure under the stimulus of acute hypoxia was also greater in the susceptible animals. Increased resistance to pulmonary blood flow was

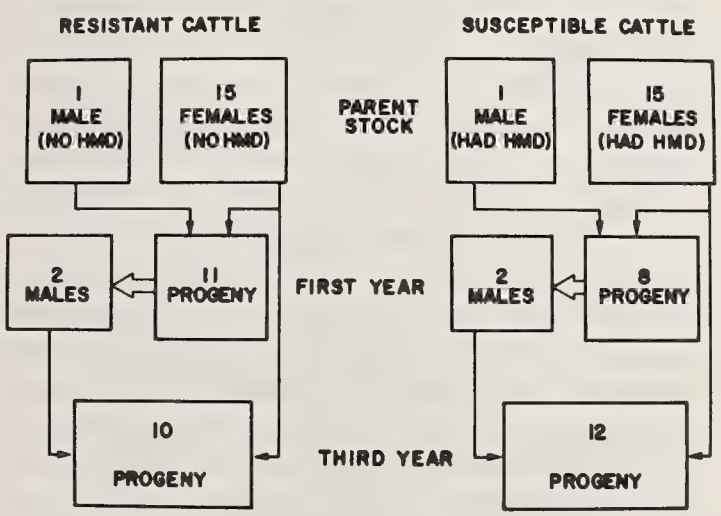


FIGURE 2.—Parent stock, breeding plan and progeny of susceptible and resistant cattle.

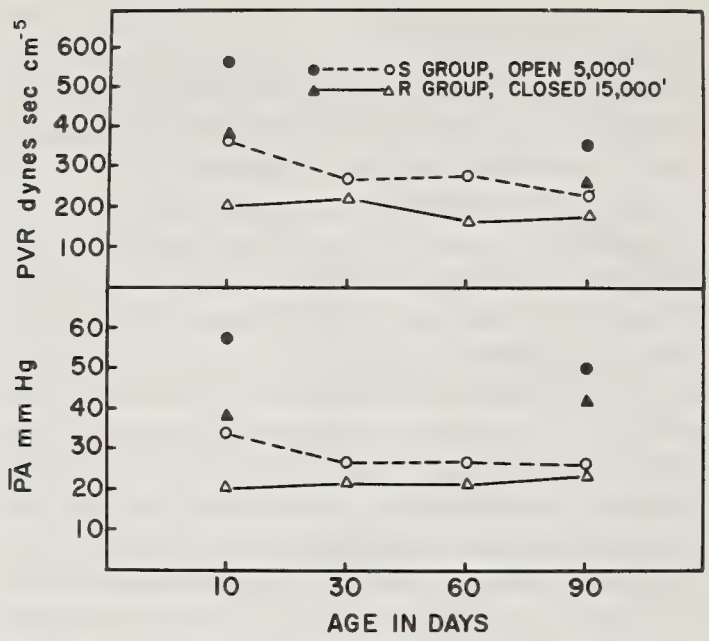


FIGURE 3.—Pulmonary arterial pressure and pulmonary vascular resistance of susceptible and resistant calves at 5,000 feet and after 2 hours at 15,000 feet altitude.

the major reason for the greater hypertension in the susceptible animals (Figure 3).

When taken to the natural altitude of 10,000 feet a similar but more dramatic difference in pulmonary vascular reactivity was observed between the two groups (Figure 4). A progressive rise in mean pulmonary arterial pressure was observed in both groups but a much greater response was present in the susceptible animals. Clinical signs of right heart failure occurred between 64–122 days at altitude in all but one of the susceptible animals and in none of the resistant animals. All of the affected animals were removed to 5,000 feet to recover with the first definitive signs of heart failure. The remaining susceptible calf developed signs of impending heart failure at 150 days at which time this animal and the entire group of resistant animals were returned to 5,000 feet. In contrast to the susceptible group, 9 of the 11 resistant animals developed only moderate levels of pulmonary hypertension at 10,000 feet, whereas the remaining two animals in this group showed substantial pulmonary hypertension but no evidence of congestive failure.

In an attempt to concentrate the genetic pool that might be responsible for resistance and susceptibility, parent females were bred to first generation males that were at the most extreme ends of the response curve for their respective groups; i.e., two males which under the challenge of altitude developed the most severe pulmonary hypertension were bred to susceptible group females while the two males which showed the lowest pulmonary vascular resistance were bred to the resistant group females (Figure 2). From these matings 12 susceptible and 10 resistant calves were produced.

Cardiorespiratory studies were carried out when the animals were five months of age at 5,000 feet altitude and in the hypobaric chamber after two hours at 15,000 feet.[12] Significant differences in pulmonary vascular reactivity were present between the two genetic groups with the susceptible calves showing greater

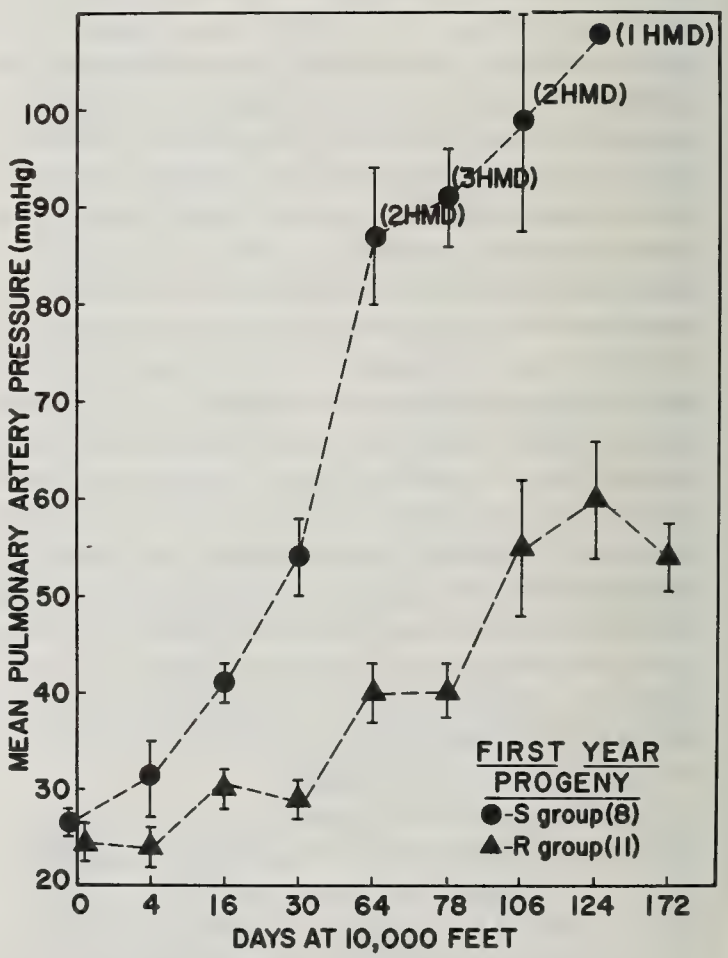


FIGURE 4.—Mean pulmonary arterial pressure in resistant and susceptible calves chronically exposed to 10,000 feet natural altitude.

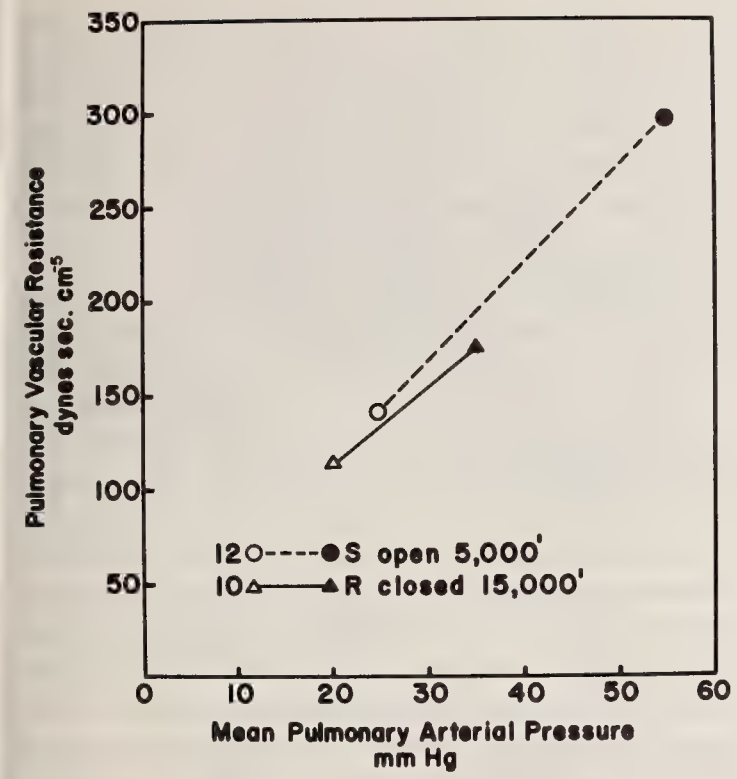


FIGURE 5.—Pulmonary vascular reactivity at 5,000 feet and after 2 hours at 15,000 feet; third year susceptible and resistant calves.

mean pulmonary arterial pressures and vascular resistance at both altitudes (Figure 5).

Five calves at the extreme ends of the response curve for both groups were selected for further study; i.e., the five calves of the susceptible group that displayed the greatest rise in pulmonary arterial pressure and vascular resistance, and the five calves of the resistant group which showed the least rise in these parameters. At nine months of age these calves were again studied at 5,000 feet altitude and after two hours at a simulated altitude of 15,000 feet in the hypobaric chamber to test whether the initial results were repeatable.[12] Again a striking difference in pulmonary vascular reactivity to hypoxia between the two groups was observed (Figure 6). Although the reactivity of the susceptible calves had decreased somewhat with age, the vascular resistance and the mean pulmonary arterial pressure was still significantly higher than in the resistant calves.

The significant differences in apparent pulmonary vascular reactivity to both acute and chronic hypoxia in both generations of calves lends support to the hypothesis that susceptibility to hypoxic induced pulmonary hypertension in cattle is at least *partially heritable.* Heritable factors probably exist in most all diseases and account for much of biologic variations observed. Inherited resistance and susceptibility to infectious diseases has been well demonstrated.[13] Of particular interest, however, in the altitude susceptible and resistant calves is the fact that the apparent genetic influence was well expressed in the initial offspring and did not require generations of genetic concentration.

The reasons for the differences in response between the two groups of cattle remain unclear. A stepwise regression analysis showed that pulmonary vascular resistance values obtained at 60 days of age at an altitude of 5,000 feet served as the best single criterion ($r = 0.632$) for predicting adaptive failure, i.e., high mountain disease in animals taken to high altitude.

Although the exact mechanism of the increased pulmonary vascular resistance in cattle as well as other animals and man has not been defined, it is generally accepted that the effec-

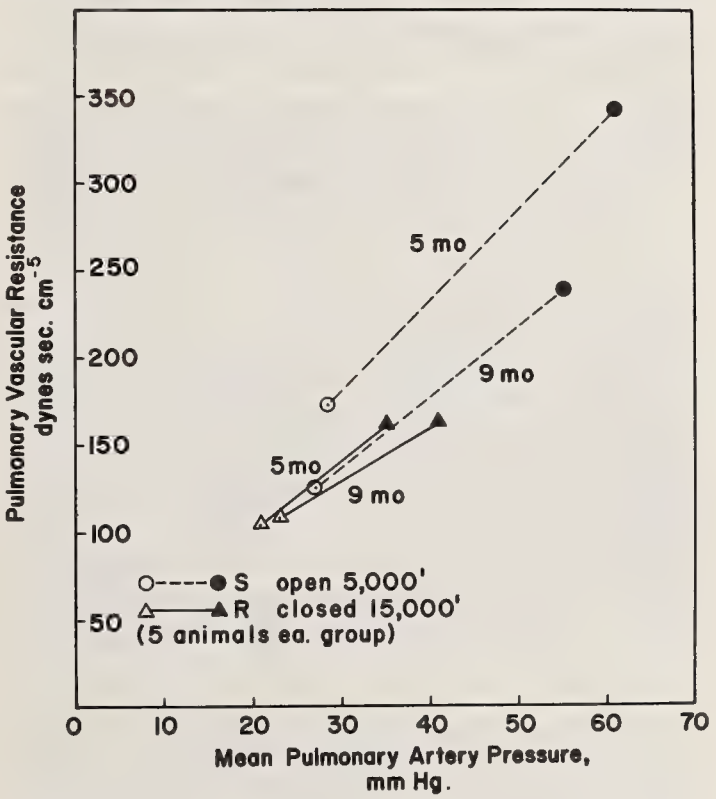


FIGURE 6.—Pulmonary vascular reactivity at 5,000 feet and after 2 hours at 15,000 feet; susceptible and resistant calves; 5 and 9 months of age.

tive stimulus is reduced oxygen tension in the distal airways or alveoli. Quite logically then, in hypoxic environments, variations in ventilation might be expected to produce individual differences in the pulmonary pressor response. Results appear to indicate that differences in ventilation may account for the different levels of pulmonary hypertension seen in the two genetic groups. Although we have not carried out ventilatory studies in these animals since they have not yet been adequately trained for this procedure, the evidence which indicates that ventilation is probably different for the two groups is the consistently lower arterial PO_2 and pH and higher arterial PCO_2 in the susceptible versus the resistant cattle (Figure 7).

We have also examined the effects of hypoxia on other systems in these animals. One system which could be expected to participate in the adaptation to hypoxia is blood oxygen transport. Closely related to this is the erythropoietic system. Interest in blood oxygen transport in hypoxic environments has increased in recent years with the discovery that in man, at least, residence high altitude or chronic hypoxia increases the level of 2,3 diphosphoglycerate (DPG) in the erythrocytes and reduces the affinity of hemoglobin for oxygen, i.e., shifts the oxyhemoglobin dissociation curve to the right.[14,15] These same parameters were studied in the two genetic groups of cattle. The two original female parent groups were the first to

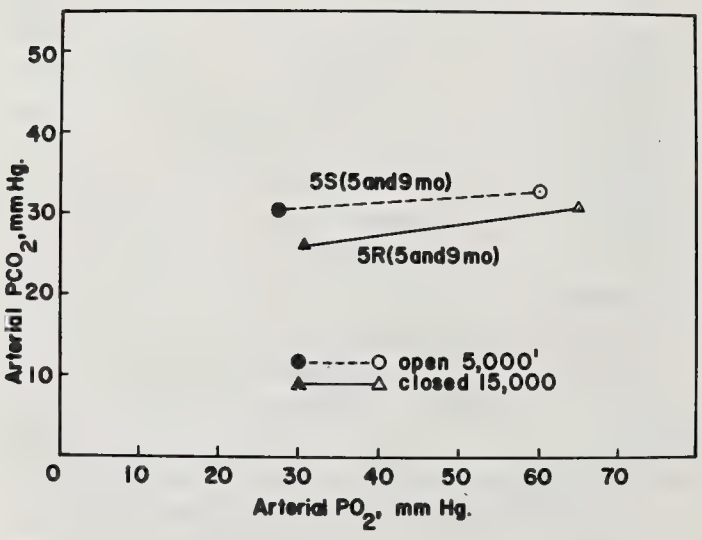


FIGURE 7.—Arterial PCO_2 and PO_2 at 5,000 feet and after 2 hours at 15,000 feet in susceptible and resistant calves.

be examined for differences in the positions of their oxyhemoglobin dissociation curves.[16] This was done, however, one year after they had been selected at high altitude and moved to the lower altitude of 5,000 feet. The results showed that the resistant females had a dissociation curve significantly more to the right.

More detailed studies of blood oxygen transport have been carried out on the five resistant and five susceptible third year offspring.[17] No significant differences were found between the two groups in the position of their dissociation curves or levels of 2,3 DPG either at the laboratory altitude of 5,000 feet or after an 18 day exposure to a chamber altitude of 15,000 feet. However, associated studies showed that the DPG levels were significantly higher in cattle permanently residing at 10,000 feet as compared with their counterparts at 1,200 feet, even though the oxygen dissociation curve of the permanent high altitude residents was significantly more to the left.

Further indication of a greater hypoxic state in the susceptible cattle was found in studies of erythropoiesis and hemoglobin. These were conducted on both the resistant and susceptible groups during chronic exposure to natural altitude of 10,000 feet and during both acute and chronic exposure to simulated altitude of 15,000 feet. Although no significant differences between the groups were noted in *erythropoietin* levels, the development of a *greater red cell* mass and longer retention of fetal hemoglobins in the susceptible group[18] may indicate a greater hypoxic stimulus in these animals.

The hallmark of the hypoxic stimulus is the development of pulmonary hypertension. In pulmonary hypertension induced by acute hypoxia and possibly in the moderate pulmonary hypertension of chronic hypoxia, the pulmonary vascular bed appears to remain labile and rapid return to normo-tensive pressures can be induced by the administration of oxygen.[9] In contrast, in the severe level of chronic hypoxic induced pulmonary hypertension, the vascular bed appears somewhat more fixed and the administration of oxygen fails to result in immediate return to normotensive levels. The intrisic reasons for the differences, if any, in these vessels remain unknown.

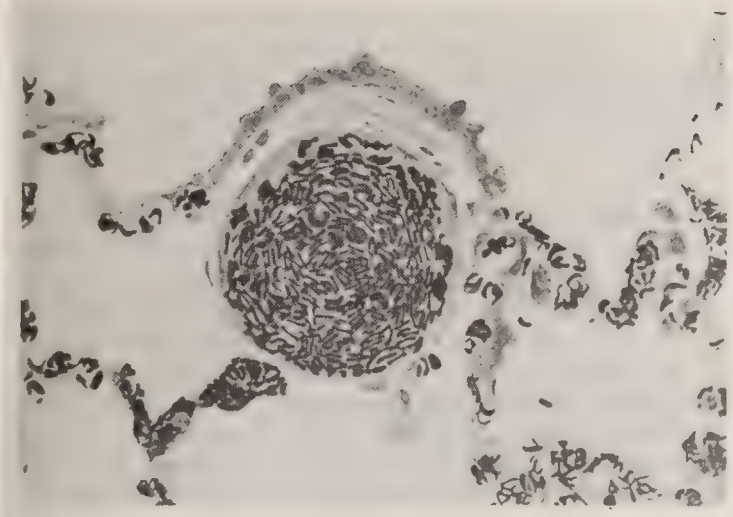


FIGURE 8.—Arteriole at level of terminal bronchiole from a normotensive animal. Media comprised of thin single layered smooth muscle. Epon section; Richardson stain × 425.

The light microscopic alterations of pulmonary hypertensive vascular disease have been described in considerable detail.[19,20] Many studies, however, have been concerned with hyperkinetic states in which the pulmonary blood flow is increased. Although the vascular lesions of hypoxic pulmonary hypertension show some similarity to these high pressure/high flow systems, the end stages of vascular necrosis, severe intimal proliferation, and plexiform formations, are seldom observed. Essentially, the hypoxic induced vascular lesions are characterized by marked medial thickening (Figures 8 and 9), moderate intimal proliferation in larger arteries and adventitial proliferation.[6,7,21,22]

A number of reports have dealt with the cellular pathology of systemic hypertension.[23-25] Our own ultrastructural investigations of small pulmonary arterial vessels in hypoxic-induced pulmonary hypertension have revealed basic similarities to the hypertensive lesions of systemic arterioles.[26] Fine structural studies provide evidence for cellular degenerative changes. A more important alteration maybe a change in the basic metabolic function of the medial smooth muscle cells as well as the endothelium, evidenced by changes in morphologic patterns of the subcellular organelle systems. Further investigations are being conducted in an attempt to relate these morphological changes to basic functional changes in hypertensive vessels.

SUMMARY

The marked reactivity of the bovine pulmonary vascular bed to chronic inhalation hypoxia make this species of distinct value as a model for studies of hypoxic-induced pulmonary hypertension. Variations in the levels of pulmonary hypertension and vascular resistance observed between individual cattle will probably ultimately be defined in functional and morphological terms. However, heredity appears to play a prominent role in governing these factors and should be considered in the interpretation of experimental results.

REFERENCES

1. WILL, D. H., and ALEXANDER, A. F. High mountain disease. In *Bovine Medicine and Surgery*, 1st ed. Am. Vet. Publications, Inc., Wheaton, Illinois, 1970.
2. HECHT, H. H., KUIDA, H., LANGE, R. L., THORNE, J. L., and BROWN, A. M. Brisket disease II Clinical features and hemodynamic observations in altitude-dependent right heart failure in cattle. Amer. J. Med. 32:171–183, 1962.
3. GLOVER, G. H., and NEWSOM, I. E. Brisket disease. Colorado Agric. Expt. Sta. Bull. 204, 1915.
4. VOGEL, J. H. K., MCNAMARA, D. G., HALLMAN, G., ROSENBERG, H., JAMIESON, G., and MCGRADY,

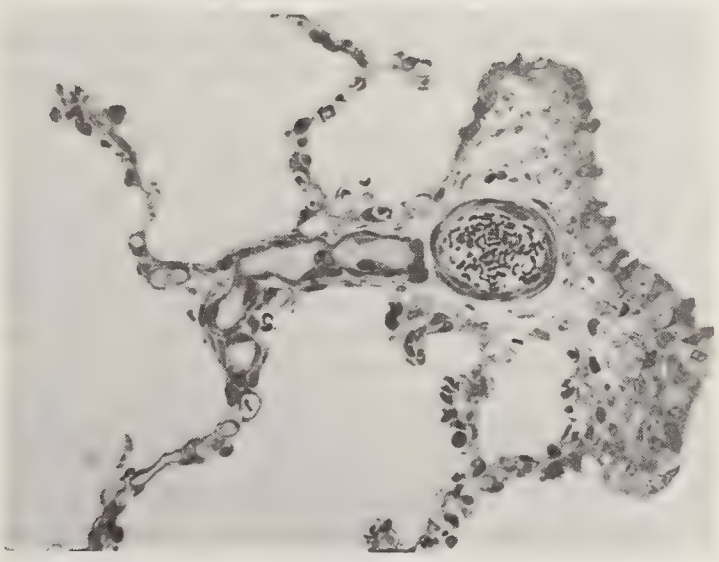


FIGURE 9.—Arteriole with small lateral branch at level of terminal bronchiole from a hypertensive animal. Note thickened media with prominent overlapping smooth muscle cells; decreased luminal size and apparent adventitial proliferation. Epon section; Richardson stain ×425.

J. D. Effects of mild chronic hypoxia on the pulmonary circulation in calves with reactive pulmonary hypertension. Circulat. Res. 21:661–669, 1967.
5. Alexander, A. F., and Jensen, R. Pulmonary arteriographic studies of bovine high mountain disease. Amer. J. Vet. Res. 24:1094–1097, 1963.
6. Alexander, A. F., and Jensen, R. Pulmonary vascular pathology of bovine high mountain disease. Amer. J. Vet. Res. 24:1098–1111, 1963.
7. Alexander, A. F., and Jensen, R. Pulmonary vascular pathology of high altitude induced pulmonary hypertension in cattle. Amer. J. Vet. Res. 24:1112–1122, 1963.
8. Will, D. H., Alexander, A. F., Reeves, J. T., and Grover, R. F. High altitude induced pulmonary hypertension in normal cattle. Circ. Res. 10:172–177, 1962.
9. Grover, R. F., Reeves, J. T., Will, D. H., and Blount, S. G., Jr. Pulmonary vasoconstriction in steers at high altitude. J. appl. Physiol. 18:567–574.
10. Will, D. H., Horrell, J. F., and Alexander, A. F. Pulmonary arterial pressure in cattle residing at different altitudes. In preparation.
11. Will, D. H., Card, C. S., Vanlandingham, G. D., and Alexander, A. F. Cardiorespiratory responses of two "genetic" types of cattle to hypobaria. Physiologist 10:347, 1967.
12. Will, D. H., Hicks, J. L., Card, C. S., and Alexander, A. F. Genetic basis for high altitude adaptation in cattle. Fed. Proc. 29:591, 1970.
13. Gowen, J. W. Experimental analysis of genetic determinants in resistance to infectious disease. Anal. N. Y. Acad. Sci. 91:689–709, 1961.
14. Eaton, J, W., Brewer, G. J., and Grover, R. F. Role of red cell 2,3-diphosphoglycerate in adaptation of men to altitude. J. Lab. Clin. Med. 73:603–609, 1969.
15. Lenfant, C., Torrance, J. D., and Finch, C. A. The regulation of hemoglobin affinity for oxygen in man. Tr. Ass. Am. Phys. 82:121–128, 1969.
16. Cueva, S., and Will, D. H. Blood oxygen transport in cattle "susceptible" and "resistant" to high mountain disease. In *Current Research in Chronic Respiratory Disease*. Proc. 11th Aspen Conf:81–86, U. S. Public Health Service, 1968.
17. Hicks, J. L. Effects of altitude on bovine blood oxygen transport. Ph.D. dissertation, Colorado State University, Fort Collins, Colorado, 1971.
18. Card, C. S. The response of the bovine erythron to hypoxic stress. Ph.D. dissertation, Colorado State University, Fort Collins, Colorado, in preparation.
19. Heath, D., and Edwards, J. E. The pathology of hypertensive pulmonary vascular disease. Circ. 18:533–547, 1958.
20. Wagenvoort, C. A. Vasoconstriction and medial hypertrophy in pulmonary hypertension. Circ. 23:740–748, 1961.
21. Arias-Stella, J., and Saldana, M. The terminal portion of the pulmonary arterial tree in people native to high altitudes. Circ. 28:915–925, 1963.
22. Hasleton, P. S., Heath, D., and Brewer, D. B. Hypertensive pulmonary vascular disease in states of chronic hypoxia. J. Path. Bact. 95:431–440, 1968.
23. Spiro, D., Lattes, R. D., and Wiener, J. The cellular pathology of experimental hypertension. Amer. J. Path. 47:19–49, 1965.
24. Gardner, D. L., and Mathews, M. A. Ultrastructure of the wall of small arteries in early experimental rat hypertension. J. Path. 97:51–62, 1969.
25. McGee, W. G., and Ashworth, C. T. Fine structure of chronic hypertensive arteriopathy in the human kidney. Am. J. Path. 43:273–299, 1963.
26. Jaenke, R. S. Ultrastructural study of hypoxic-induced pulmonary hypertension in the bovine. Ph.D. dissertation, Colorado State University, Fort Collins, Colorado, 1969.

DISCUSSION

J. T. Reeves, University of Kentucky, Lexington, Kentucky: Many of us have awaited these findings with considerable interest. It is very nice to see the results of this study of bovine families at high altitudes. The possibility that the pulmonary hypertension relates to hypoventilation, as opposed to increased reactivity of the pulmonary vessels, could be decided very nicely if the PO_2, perhaps even the alveolar PO_2, were kept constant given hypoxic stimulus. I wonder if you have any idea about that?

Dr. Alexander: No I don't.

Gerald Moss, Rennselaer Polytechnic Institute, Troy, N.Y.: Along the lines that were just brought up, Dr. Alexander, we've been working with calves breathing room (ambient pressure) air; but with an isolated brain percussion with a hypoxemic profusion (simulating acutely very high altitudes, about 30–35 mm. of mercury oxygen), and within minutes we get pulmonary arterial hypertension. We also get severe lung changes, but we get a reactive thing which appears to be central neurogenic.

I was interested in those electron micrographs and I'm not sure I understood. Is it true that you think that the animals which are hyper-responders have evidence of more damage in the organelles? Can you explain that a little bit more?

Dr. Alexander: No, not as a difference between the groups. It was as a difference between the normal tensive animal in the group.

A MODEL FOR CLOSED THORACIC OBSERVATION OF THE PULMONARY MICROCIRCULATION IN SHOCK

W. A. Cook, P. B. Speiller, H. Sherman and S. K. Klausner*

The design, preparation, and use of a lucite membrane thoracic window for incident-light, dark-field, cinemicroangiology is presented. The usefulness of this method of *in vivo* microvascular study for solid and transparent organs during low-flow states is described.

INTRODUCTION

It has been pointed out by several authors that physiologic study of the pulmonary microvasculature is best performed through an airtight thoracic window.[2,3,8] Ingenious devices have been developed to this end for over 50 years.[3,5,7,8] Most recently an extremely nonreactive window has been described by Krahl consisting of a double flanged lucite bobbin and a window of 0.5 mil FEP teflon film stretched over the inner surface of the bobbin.[4]

We are using a method of microsvascular investigation that employs the metallurgical microscope. A set of epiobjective lenses are coupled with the system to deliver light through the microscope itself. This light which obviates the need for tissue transillumination can be delivered in a bright or dark-field mode. We have found dark-field illumination most satisfactory. The new method of microscopic technique required our modifying the thoracic window and the support of the microscope.[1]

METHOD

First of all we wished to observe any gravity related intravascular phenomena and therefore decided to arrange our equipment to view the vessels from the horizontal aspect (Fig. 1). This required a special mount carrying the light source, microscope, and cameras (still and cine). This mount is so arranged that this 40 to 50 pounds of equipment all moves with the microscope when either its coarse or fine focus is changed. We utilize a xenon burner which provides light at 6000° Kelvin and has roughly the same spectrum as sunlight.

For open thoracic work in acute experiments we use a pylon containing a large diameter cover slip against which the organ is pressed for observation. However, as pointed out, physiologic study of the pulmonary vasculature is best done through an airtight thoracic window.[2,3,5,7,8] The mount shown in Figure 1 is a part of our system for study.[2,6] The basic requirements in construction of a new thoracic window adapted to this system of incident dark-field illumination were three. First, any interposed material between the tissue and the objective lens must have a high degree of polish at its air or outer interface. This is essential in order to prevent back-scatter of the incident light which in turn reduces tissue illumination. The second requirement is that this interposed material be plane to a surface perpendicular to the axis of observation. This assures minimal distortion and more even tissue illumination. The last requirement is limitation of tissue motion. Peculiar to this high magnification (450X), incident-light system is an optical tolerance to slow lateral tissue motion. Marked blurring of the field of view only occurs when there is motion along the axis of observation, and we therefore sought to eliminate this motion.

We were interested in developing windows which met these three requirements and were also suitable for study of various size laboratory animals. Our point of departure was Krahl's lucite bobbin-teflon membrane window.[4] This design failed to provide limitation of motion along the axis of observation due to the flexible teflon membrane, or a means by which the window could be rigidly stabilized at right angles to that axis.

Our first modification was to mill a thread

* Albert Einstein College of Medicine, Bronx, New York.

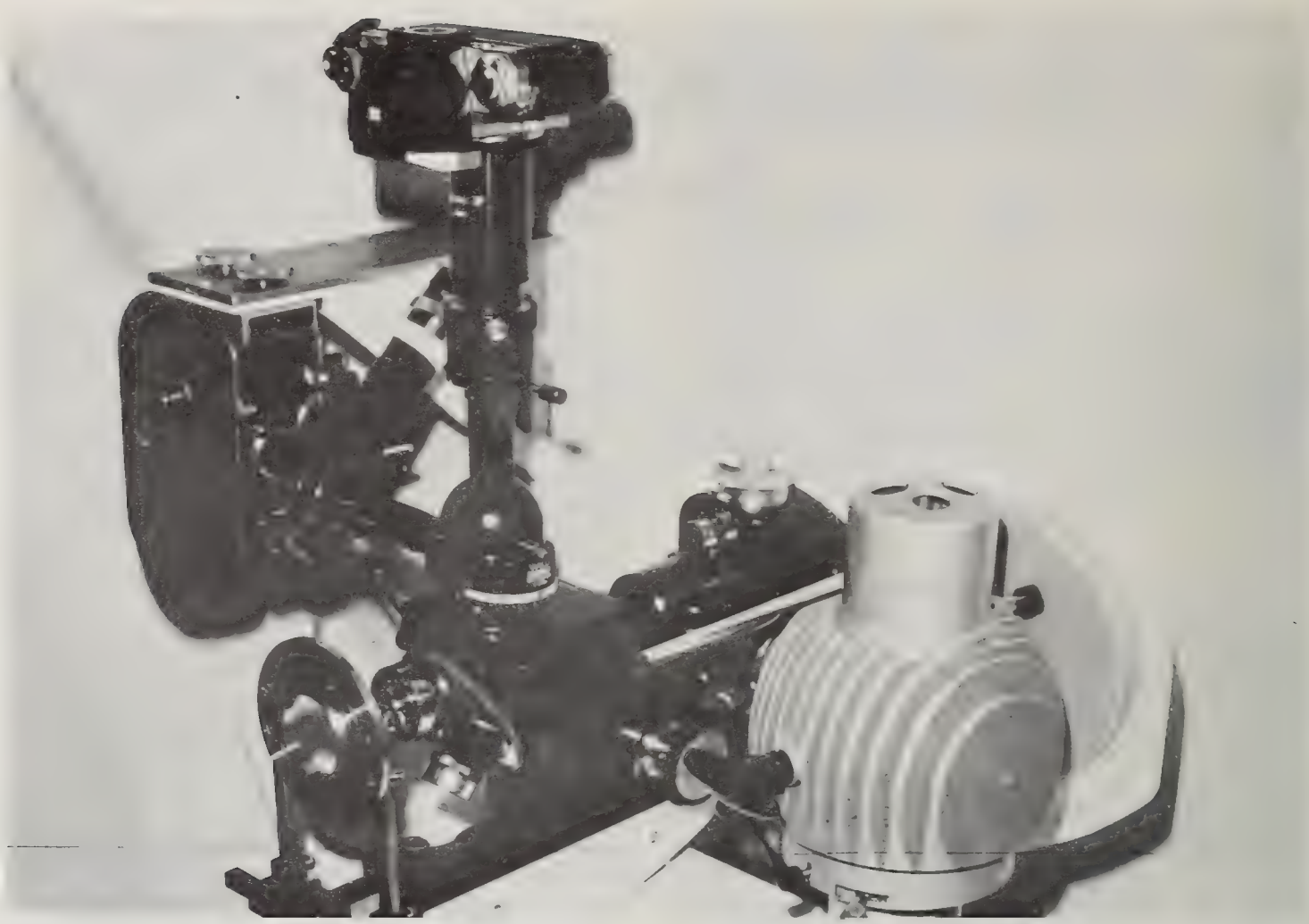


FIGURE 1.—Incident light dark-field microscope with xenon light source and photographic attachments. Note position of thoracic window support.

into the inner surface of the bobbin (Fig. 2). A stainless steel supporting device was then constructed which screwed into the bobbin and was in turn anchored to vertical support bars mounted on our microscope (Fig. 3). The window was thus held motionless, and its plane perpendicular to the axis of observation. The steel support is adaptable to any size window since its central portion is removable and can be replaced by one of an appropriate size (Fig. 4). A secondary benefit was achieved by the addition of the screw thread. One of the recurring problems with chronic thoracic windows is membrane damage caused by the animal or its environment. A rigid lucite cover was made that could be securely screwed into the threaded bobbin and this prevented that damage (Fig. 2).

The final problem, lung motion in the axis of observation, was overcome by utilizing a highly polished $\frac{1}{32}$ inch rigid lucite plate as the window element instead of flexible 0.5 mil teflon. This material is far easier to handle, more durable and allows motion only in a lateral plane. We continue, however, to protect this lucite membrane from the animal's daily activities with the thick outer cover.

RESULTS

This new window has been successfully left in place for three weeks (Fig. 5). Tissue reaction has been minimal. Optically, light transmission is on a par with FEP teflon. There is minimal back-scatter and no appreciable visual distortion. Color rendition is uniformly excellent. We can now view the lung during active respiration in chronic animals breathing on their own (Fig. 6).



FIGURE 2.—Modified bobbin-like intrathoracic window showing screw thread and rigid removable cover.

The use of the incident-light, dark-field system in conjunction with the modified thoracic window, permits microscopic study of the lung under truly physiologic conditions. We have minimized extraneous tissue motion, eliminated the need for the additional surgery required in transillumination techniques, and still provided the necessary light intensity for high magnification studies. We feel that this new system is a superior method for studying the pulmonary microstructure in the living animal.

Our immediate objective in development of this model was to observe changes occurring in the pulmonary microcirculation of animals in shock. We have been frustrated in this objective by the discovery of a number of factors which alter pulmonary blood flow other than shock. Accepting this diversion, we have studied these factors in order to have a more nearly normal model that will allow the discrimination of the subtle changes due to shock.

Beginning with acute studies in open thoracic preparations we have found that exposure to air and drying will produce changes in the pulmonary capillary blood flow of normal animals after about one hour. This would seem to be the time limit for acute observations if they are to be reliable.

In animals equipped with the thoracic window a number of additional factors which alter pulmonary blood flow have been found. The first of these is rapid administration of normal saline. Infusion of physiologic saline equal to 10% of normal plasma by a Harvard pump at a rate of 30 ml/min. will cause rapid aggregation of erythrocytes and plugging of pulmonary capillaries by these aggregates. This phenomena is also seen in shock. Thus, careful attention



FIGURE 3.—Lucite window in place on support apparatus.

to surgical technique must be observed to prevent blood loss and replacement during preparation of the model if it is to be valid.

Trauma to the lung surface during insertion of the window must be carefully avoided as this caused interstitial edema and even hemorrhage which could be interpreted as due to the effect of shock. For chronic animal preparation this is doubly important as trauma sets up a pleural reaction that prevents later observation.

Positive pressure ventilation is another factor which affects pulmonary blood flow. We have observed that respiratory rate, tidal volume, end expiratory pressure and ventilatory cycle all affect flow. Increased rates cause decreased blood flow. Excessive tidal volumes impede flow as well. The most normal flow on positive pressure ventilation is obtained with ventilatory volumes and rates close to the normals set forth in The Biological Handbook of Respiration and Circulation.[1]

If a phase adjustment is available on the ventilator it is best to set it so that the ventilatory cycle is ⅓ inspiratory and ⅔ expiratory as this gives the most normal blood flow. Finally the exhalation part of the ventilator should be placed under 5 cm of water to maintain a positive end expiratory pressure. This is more effective at preventing atalectasis and maintaining a normal function residual capacity than intermittant "sighs" of high pressure, and gives less change in the pulmonary circulation. Obviously the best preparation is a mildly sedated animal with a chronically implanted window and breathing on its own. Despite these considerations we wish to underline the suggestion that a careful survey of vascular changes always should be made in the control period.

SUMMARY

The use of the incident-light, dark-field system in conjunction with this newly modified thoracic window, permits microscopic study of the lung under truly physiologic conditions. We have minimized extraneous tissue motion, eliminated the need for additional surgery required in transillumination techniques, and still provided the light intensity for high magnification studies. We feel that this new system is a superior method for studying the pulmonary microstructure in the living animal, and particularly in the rapidly changing vascular states following shock. The limitations and precautions of the system must, however, be observed if valid data are to be obtained. Finally we wish to point out that with the incident-light technique it is possible to study changes in flow in many organs beside the lung. We have been able to photograph the microcirculation of the brain, liver, kidney, and gut with a satisfactory degree of resolution at magnifications to 450X. We therefore feel that this system can be recommended to investigators in a wide area of study concerning the response of laboratory animals to low-flow states.

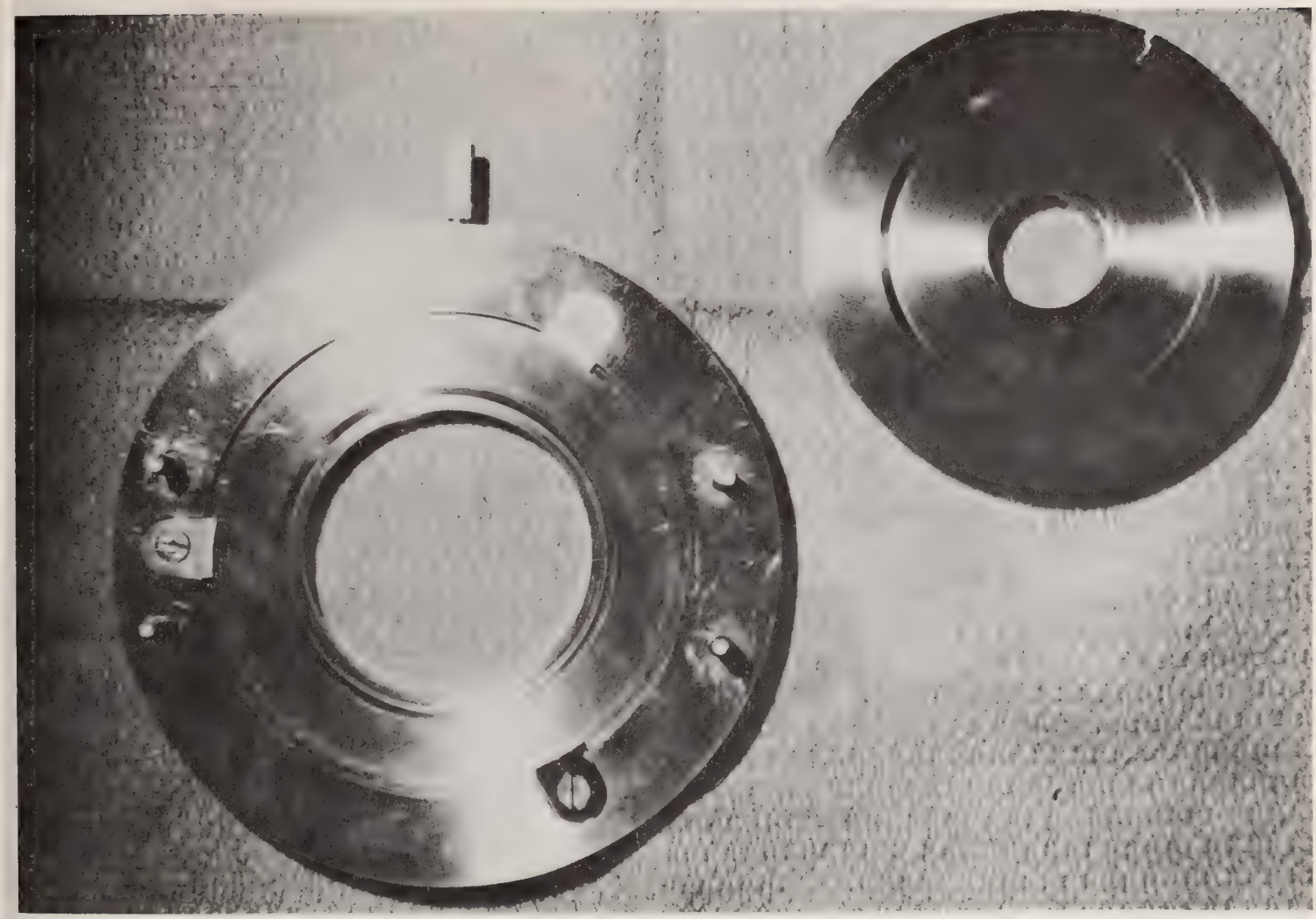


FIGURE 4.—Stainless steel support with interchangeable center for different size windows.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Miss Adele Giro.

REFERENCES

1. ALTMAN, P. L. and DITTMER, D. S. (Eds.) Respiration and Circulation, Federation of American Societies for Experimental Biology, Bethesda, Md., 1971.
2. KLAUSNER, S. K., SHERMAN, H., and COOK, W. A. A Thoracic Window for Incident Light Microscopy. Vasc. Surg. 5:215, 1971.
3. KRAHL, V. E. A method of studying the living lung in the closed thorax, and some preliminary observations. Angiology, 14:149, 1963.
4. KRAHL, V. E. *In vivo* microscopy of the rabbit's lung. In *Second European Conference on microcirculation*. Ed. H. Harders, Bibl. Anat. Fasc. 4:400, 1964.
5. MACGREGOR, R. G. Examination of the pulmonary circulation with the microscope. J. Physiol. 80:65, 1934.
6. SHERMAN, H., KLAUSNER, S. K., COOK, W. A. Incident dark-field illumination: A new method for microcirculatory study. Angiology, 22:295, 1971.
7. TERRY, R. J. A thoracic window for observation of the lung in a living animal. Science, 90:43, 1939.
8. WEARN, J. T., ERNSTENE, A. C. and BROMER, A. W. The behavior of pulmonary vessels as determined by direct observation in the intact chest. Am. J. Physiol. 85:410, 1928.



FIGURE 5.—Thoracic window in dog. Lung fissure and inflammatory pleural vessels grossly visible.

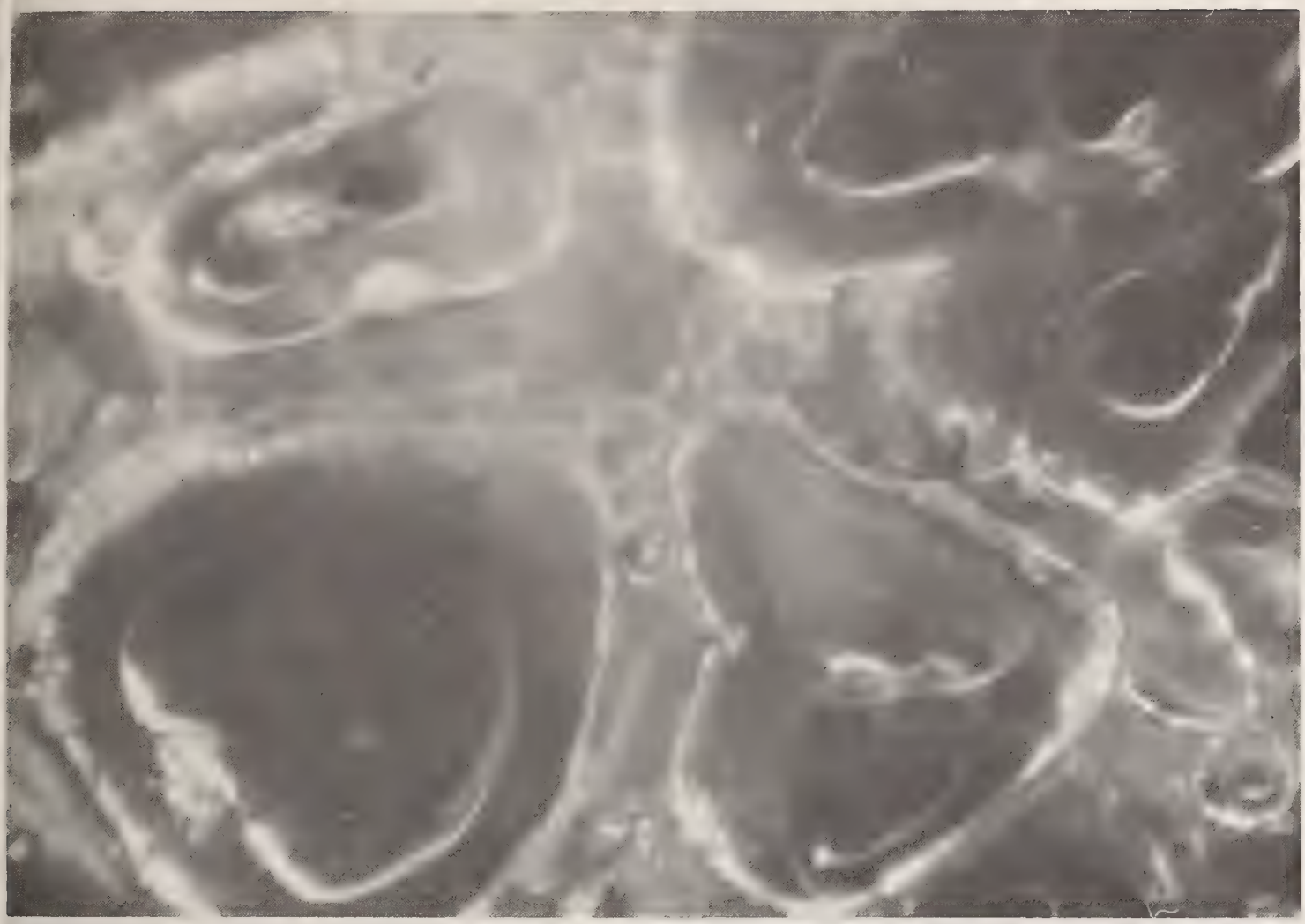


FIGURE 6.—Photomicrograph of lung through the thoracic window showing alveoli and perialveolar capillaries.

A PULMONARY XENOGRAFT MODEL FOR MICROCIRCULATORY OBSERVATION

W. A. Cook, P. B. Speiller, S. K. Slausner, S. B. P. Sinha, Y. Kikkawa and F. J. Veith*

Several pulmonary xenograft models have been investigated to determine their usefulness in microcirculatory study of hyperacute rejection. Pig, sheep and cat lungs were transplanted to the dog. Our microcirculatory observations were made with an incident light, dark-field technique employing a metallurgical microscope. This allows observation of the pulmonary vasculature through the surface of the intact lung. The most suitable of these models was a cat-to-dog pulmonary xenograft utilizing both cat lungs as a lung block grafted into the left thorax of the dog.

INTRODUCTION

Xenograft models should have ready availability of the animal donor and recipient and ease of technical preparation. A cat-to-dog lung graft model, which we have recently developed and which consists of a transplant of both cat lungs into the left hemithorax of a dog, meets these requirements.

METHODS

The donor cats were anesthetized with intraperitoneal pentobarbital and their tracheas intubated via a tracheostomy. The animals were placed on a volume controlled ventilator, and their chest opened by median sternotomy. The main pulmonary artery was dissected free to its root. The superior and inferior venae cavae were ligated. Rapidly thereafter, the trachea was clamped and obliquely transected, and the heart and both lungs excised as a block after dividing the aorta. The ventricles were removed from the lung block by incising the atria circumferentially just proximal to the atrioventricular junction and by transecting the main pulmonary artery just distal to the pulmonary valve. The left atrial appendage and excess portions of the right atrium were excised so that a circular cuff of left atrium containing the orifices of all the pulmonary veins was left attached to the lung block. A cannula was tied loosely in the main pulmonary artery, and all cat blood was removed from the lung block by perfusing the vascular tree with chilled (4°c) Ringer's lactate solution at a pressure of 15 cm of H_2O until the effluent coming from the pulmonary veins was clear.

While this was being done, the recipient dog, which was also anesthetized with pentobarbital, was given 2 mg/kg of heparin, and ventilated with a volume respirator, and a left pneumonectomy was performed. The left pulmonary artery, the left mainstem bronchus, and a cuff of left atrium containing the orifices of the pulmonary veins to the left lung were occluded with atraumatic clamps. Care was taken to preserve as much of these structures as possible.

The cat lung block was then placed in the left hemithorax of the recipient dog (Fig. 1). The lung block was held anteriorly and the posterior wall of its left atrial cuff was sutured to the recipient left atrial cuff with a continuous everting 6–0 silk mattress suture which was reinforced by an over-and-over suture of 6–0 silk. The obliquely cut trachea of the cat lung block was then sutured to the end of the dog left mainstem bronchus with a continuous suture of 4–0 dacron, and the bronchial clamp was removed so that the lung block was again ventilated. The lung block was then held posteriorly and the anterior wall of the left atrial anastomosis completed with continuous everting and over-and-over sutures. The main pulmonary artery of the cat was then anastomosed to the left pulmonary artery of the dog with a continuous over-and-over suture of 6–0 silk, and the vascular clamps removed. The cat lungs were without ventilation for from 25–35 minutes and without circulation for from 50–60 minutes.

Of primary importance to our work is the

* Albert Einstein College of Medicine, and Montefiore Hospital, New York, New York.

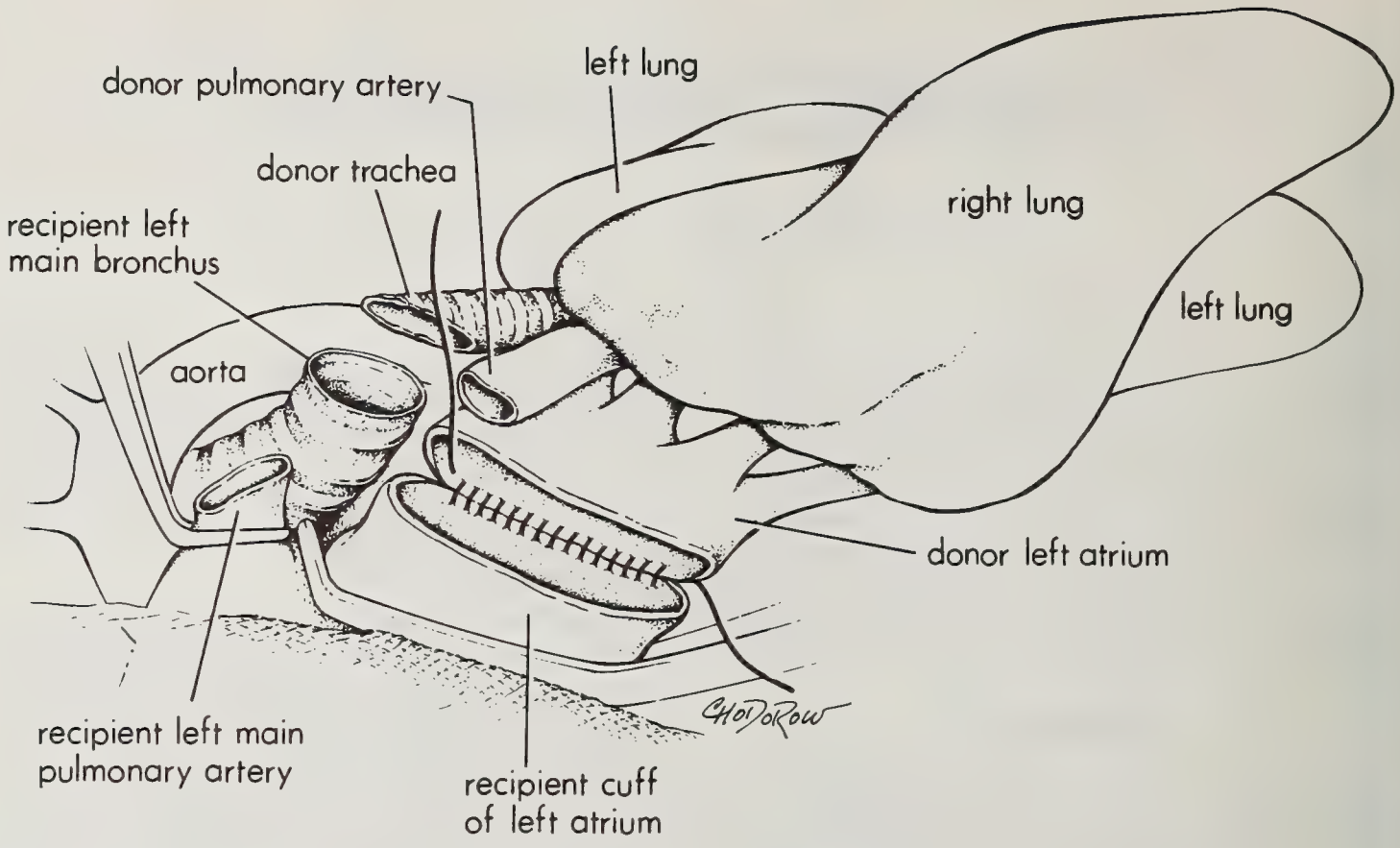


FIGURE 1.—View of cat total lung block being anastomosed to left pulmonary hilus of a dog. The posterior wall of the cat's atrial cuff has been sutured to the dog's atrium and the transplant reflected posteriorly so that the atrial anastomosis can be completed, the pulmonary arteries anastomosed, and the cat trachea sutured to the end of the dog's left main bronchus.

basic structure of the lung being observed. We are utilizing a metallurgical microscope with epi-objective lenses for *in vivo* study of the pulmonary vasculature during hyperacute rejection.[9] This microscope delivers incident light to the lung thereby eliminating the need for transillumination. We use this microscope to deliver the light in a dark-field mode which gives a side and back lighting effect, and a didymium filter which makes the erythrocytes highly visible. With this equipment we can record intravascular events on Kodachrome II film at film speeds up to 64 frames/second and 450× magnification. The one factor that prevents such observation is a dense collagenous pleura. The cat and dog do not have such pleurae and are therefore highly suitable as well as readily available. Sheep have dense subpleural collagen and are not suitable, and pigs have a normal pulmonary pressure 15–20 mm Hg greater than the dog and are therefore unsuitable.

RESULTS

We have observed canine autografts, cross-sensitized canine allografts, and cat-to-dog xenografts successfully.[2] Of particular interest are the observations made in the cat-to-dog pulmonary xenografts. In this model, photography was begun with the cat lungs washed free of cells with cold, heparinized Ringer's lactate solution and transplanted, but before the dog's circulation to these lungs was established. When the vascular clamps were opened and flow began, erythrocytes were immediately involved in dense aggregates. The aggregates appeared to form in the time required for passage from the dog's plumonary artery to the cat's alveolar capillaries i.e. in less than one minute.

These erythrocyte aggregates did not adhere to the vascular walls but appear to jostle along in the vascular flow. However, they did adhere to each other and acted as large mechanical

obstructions to the microcirculation. As the process progressed, the size of the red cell aggregates increased so that progressively larger arterioles became plugged. Eventually all flow in these vessels stopped. The venous side of the circulation showed evidence of plasma streaming and acellularity suggesting that the red cell aggregates were becoming caught in the arterioles and capillaries. The dogs were heparinized and intravascular thrombosis was not observed *in vivo* or in the fixed microscopic sections.

The rejected lungs were grossly heavy and dark red. The degree of involvement varied in a mosaic distribution similar to the normal subtotal perfusion pattern of lungs in resting animals.[1] On fixed section, the changes were most marked in those parts of the lung where flow was initially greatest. Serial biopsies revealed the sequence of events shown in figures 2–5. Immediately after the cat lungs were first perfused with dog blood, the perfused areas were found to be massively engorged with erythrocyte aggregates (Fig. 3). These aggregates filled the alveolar capillaries and small arterioles. Often these areas of severe erythrocyte aggregate engorgement were adjacent to areas that were completely normal. By ten minutes this congestive process was widespread and became more pronounced. Interstitial and intra-alveolar edema were seen at this time (Fig. 4). After 15 minutes, the capillary engorgement had reached its maximum extent and severity. In many cases when this was severe, it was associated with atelectasis. At this stage, interstitial and intra-alveolar hemorrhage were seen. Despite the marked engorgement of the capillaries, the veins contained only plasma with

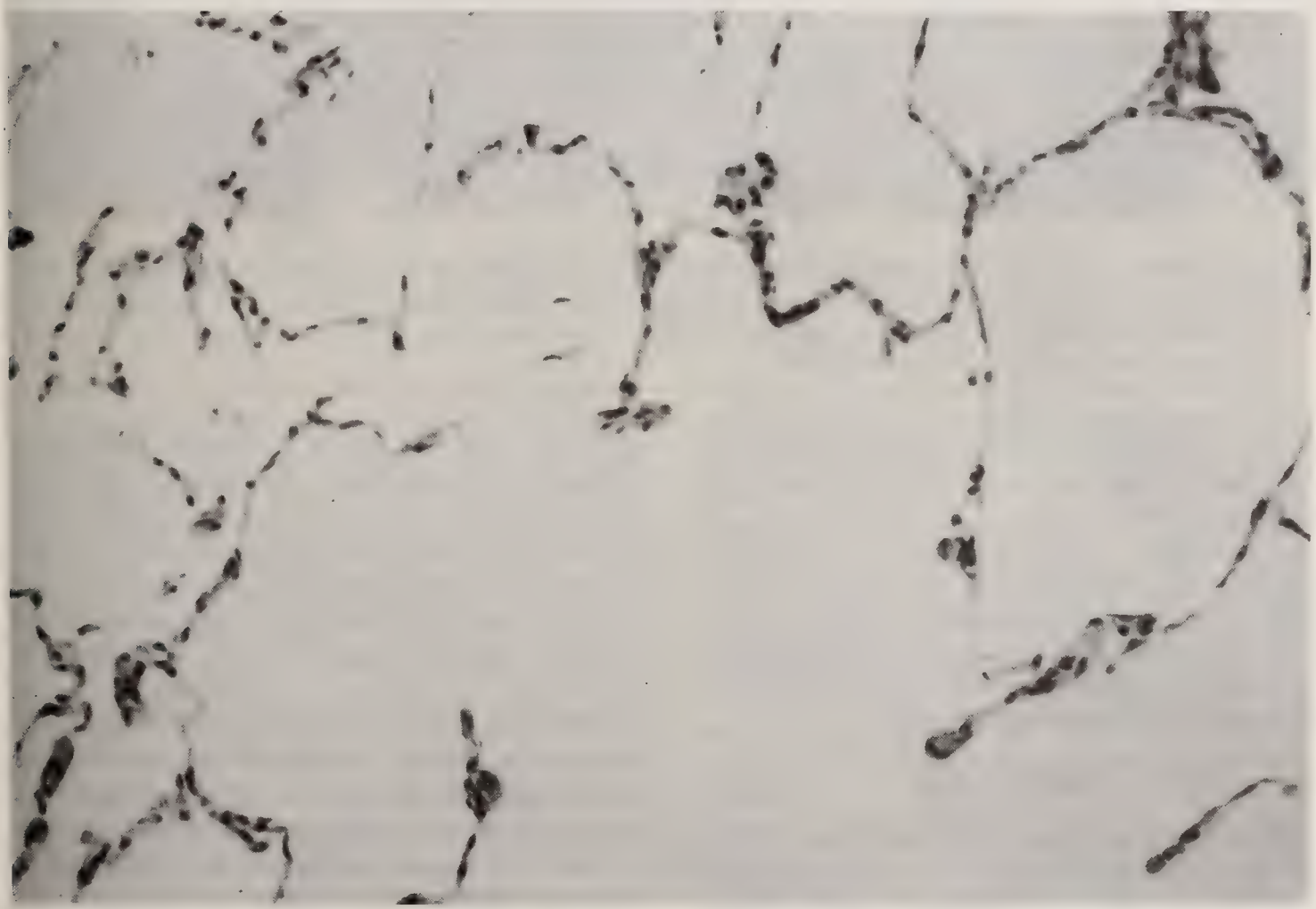


FIGURE 2.—Normal cat lung showing thin alveolar septa with a few isolated erythrocytes (arrow) and occasional polymorphonuclear leucocytes and monocytes. This biopsy was taken before dog blood entered the cat lung.

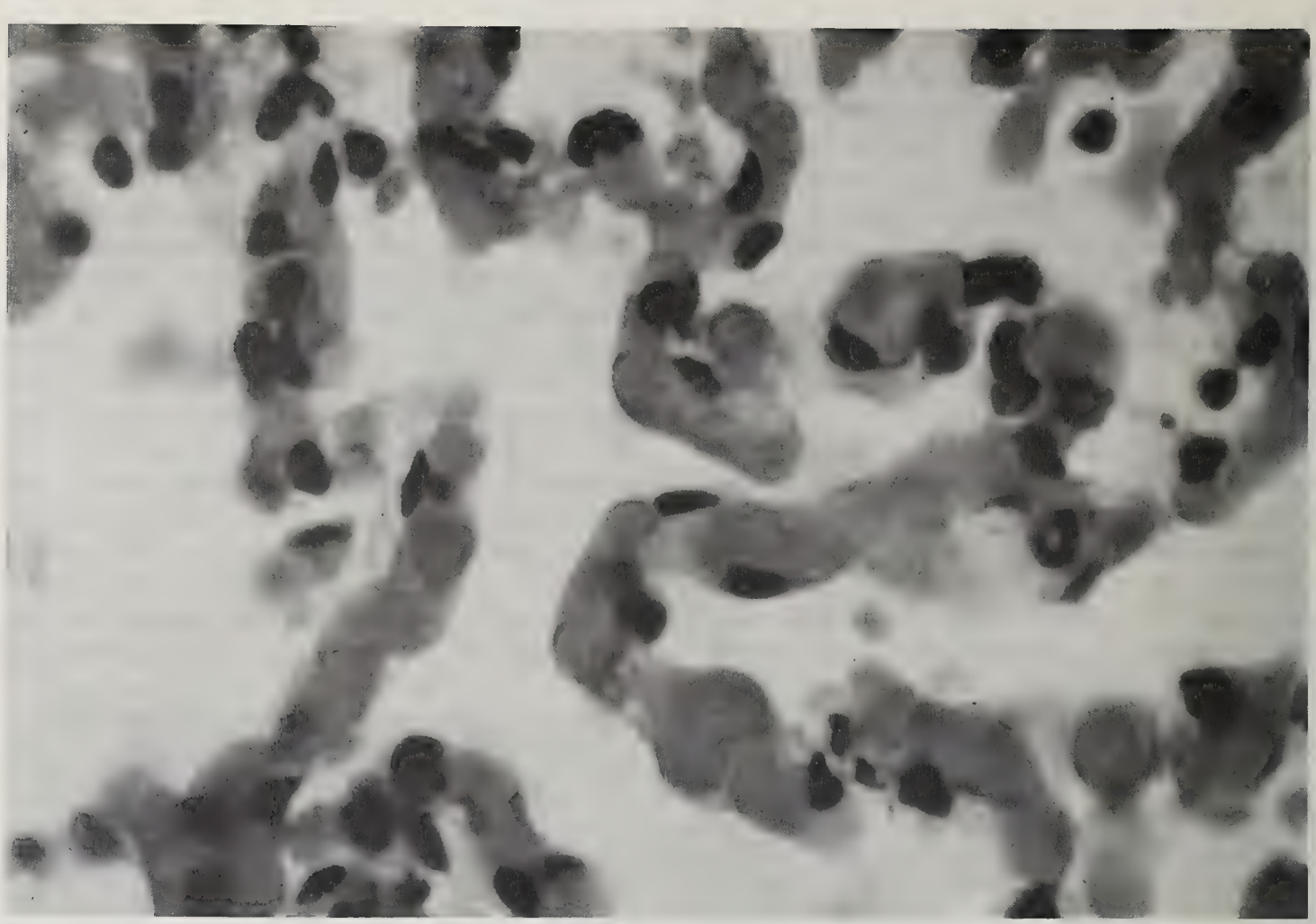


FIGURE 3.—Cat alveolar capillaries after onset of perfusion in a dog. There is extreme capillary congestion with packed red cell masses appearing as bead-like projections. Occasional polymorphonuclear leucocytes are seen, but they are never seen in groups or increased numbers. This biopsy was taken two minutes after the dog's circulation was established to the cat's lung.

very few if any blood cells (Fig. 5). Leukocyte and platelet aggregates were not found in the erythrocyte plugs.

In addition to observations in the transplanted lung, we have made observations in cat-to-dog renal xenografts. Here the same type of early intravascular erythrocyte aggregation was seen occurring in cortical capillaries located between distal convoluted tubules.

DISCUSSION

Hyperacute rejection can occur with widely divergent xenografts and when allografts are transplanted into a previously sensitized recipient who has circulating antibodies to the graft.[3,11] This process leads to rapid cessation of blood flow and destruction of the transplanted organ. To better understand this phenomenon, it is necessary to delineate its progress and causes. It is also important to reconcile some of the discrepant histologic findings reported in the literature. The present study was, there fore, undertaken to provide a morphologic examination of the developing pathology *in vivo*.

Although a large literature exists on hyperacute rejection in kidneys, we are unaware of any description of this process in the lung.[3,10,11] Our study shows that pulmonary hyperacute rejection, like hyperacute renal graft rejection, is charcterized by a rapid cessation of graft blood flow. In contrast to hyperacute renal rejection, however, it appears that hyperacute rejection in the lung is, at first, a focal phenomenon. This

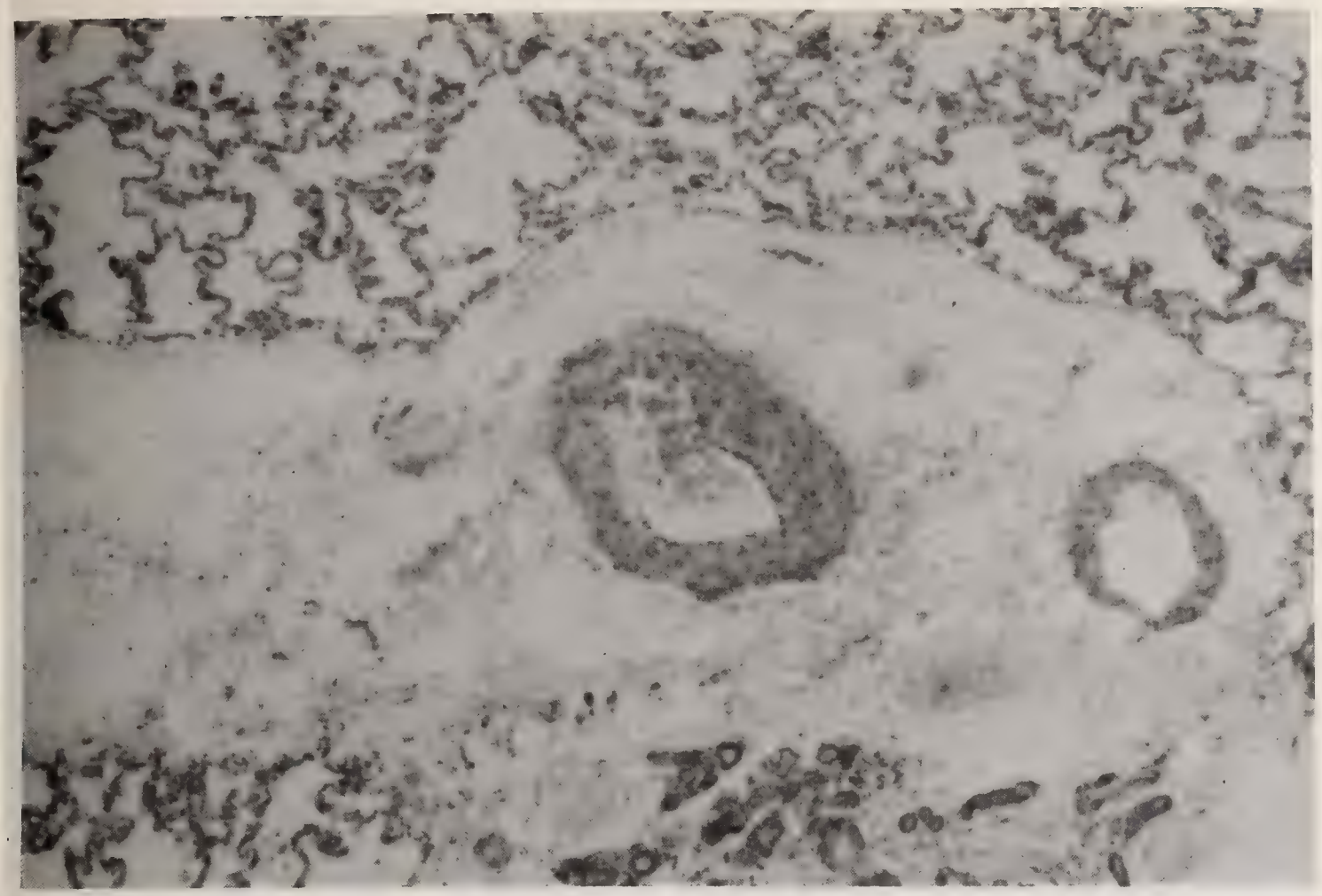


FIGURE 4.—After ten minutes, focal congestion has become diffuse and marked. Although no atelectasis has occurred, there is both interstitial and intra-alveolar edema.

is probably related to the subtotal perfusion pattern which normally occurs in the lung.[1] Hyperacute rejection of renal allografts has been characterized by the presence of congestion, endothelial distruction, focal vascular thrombosis, hemorrhage, and platelet and leukocyte plugging of capillaries, arterioles, and occasionally venules.[5,7,8,11] On the basis of our study, it would appear that the primary event in hyperacute rejection of the lung is erythrocyte aggregation. This aggregation seems to be an important factor in causing the cessation of blood flow, and in our studies was noted before any other alterations in the microsvasculature could be observed either by histologic study or by *in vivo* microscopy. Although preformed recipient antibodies have been implicated in other forms of hyperacute rejection, it is difficult to see how these antibodies could lead directly to the erythrocyte aggregation in the xenograft models in our studies.[4,10] All donor blood was removed from our xenografts prior to their transplantation. Therefore, after establishment of the recipient circulation to the xenograft, only recipient antibody could be present in large amounts. For this to agglutinate recipient red cells, it must first damage some element of the donor lung and we observed no morphologic evidence of injury to graft structures such as endothelium early in the hyperacute xenograft rejection process.

The red cell aggregation was not modified by heparinization, and thrombosis was not observed. Thus, simple intravascular coagulation was not a primary event in the hyperacute rejection process in our studies. Since neither erythrocytes nor their aggregates adhered to

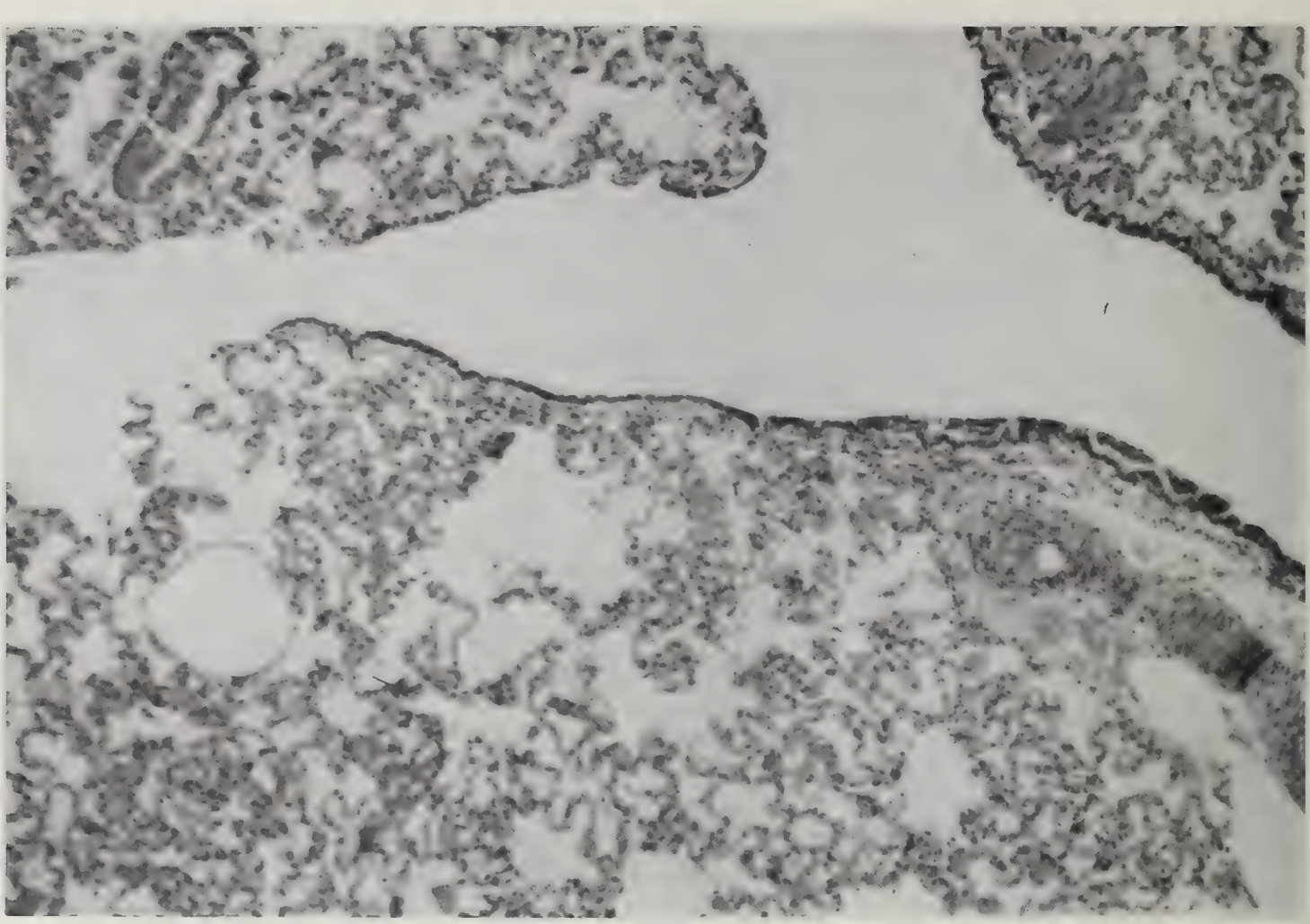


FIGURE 5.—After fifteen minutes, there is massive capillary and precapillary engorgement with partial occlusion of small arterioles by erythrocyte aggregates. The veins are distended with plasma, but nearly devoid of cells. There is also marked atelectasis, and interstitial and intra-alveolar hemorrhage.

the vascular endothelium, our studies do not implicate primary endothelial damage in the process although it could have occurred. What we saw was a very rapid mechanical plugging of capillaries and then arterioles by the dense erythrocyte aggregates. This was followed, as rejection progressed, by interstitial and intra-alveolar edema, hemorrhage, and atelectasis. In no instance, either on *in vivo* microscopy or histologic examination, could we find evidence that either platelets or leukocytes were initiating the process. Although it is possible that small numbers of platelets may be surrounded by the erythrocyte aggregates, this was not demonstrated by light microscopy.

Of particular interest were our previously reported studies of lungs in intact cats after dog blood or plasma infusion.[2] Infusion of whole dog blood into the cat produced changes in the cat lungs on *in vivo* microscopy and histologic examination identical to those seen in xenografted cat lungs. Almost identical changes occurred when cell-free dog plasma was infused into the cat lung. When the dog plasma was first absorbed repeately with cat erythrocytes, the process did not occur. After an hour there were essentially no intravascular changes. This is consistent with the findings of Linn, Moberg and their co-workers that absorption of xenogeneic plasma by donor red cells or antigen delayed the manifestations of hyperacute rejection.[4,6] The findings also suggest that anti-cat antibody initated the erythrocyte agglutination process in our infusion model.[2] In addition, our infusion studies show that the presence of a foreign erythrocyte is not necessary for the

process to occur although the xenograft studies reported herein show that foreign erythrocyte agglutination plays a major role in lung xenograft rejection per se. The apparent importance of this agglutination reaction in xenograft rejection makes it imperative that a better understanding of its origins and underlying mechanism be sought.

SUMMARY

Using this model we have made *in vivo* observations which suggest that massive erythrocyte aggregation and mechanical plugging of capillaries is the earliest event in hyperacute rejection. Early studies of renal xenograft rejection suggest a similar process occurs.

ACKNOWLEDGMENT

We would like to acknowledge the technical assistance of Adele Giro, Michael Torres, and Israel Colon.

REFERENCES

1. Cook, W. A., Baxter, C. R., and Ferrell, J. A. Pulmonary circulation after dermal burns. Vasc. Surg. 2:1, 1968.
2. Cook, W. A., Klausner, S., Sinha, S., Kikkawa, Y., and Veith, F. J. A new look at hyperacute rejection. Ann. Thor. Surg. 13:388, 1972.
3. Egdahl, R. H., and Hume, D. M. Immunologic studies in renal homotransplantation. Surg. Gynec. Obstet. 102:450, 1956.
4. Linn, B. S., Jensen, J. A., Portal, P., and Snyder, G. B. Renal xenograft prolongation by suppression of natural antibody. J. Surg. Res. 8:211, 1968.
5. Marceau, J. P., Hallenbeck, G. A., Zollman, P. E., Butler, H. C., and Shorter, R. G. A comparison of autoplastic, allogeneic and xenogeneic perfusion of isolated kidneys. J. Surg. Res. 5:492, 1965.
6. Moberg, A. W., Shons, A. R., Gewurz, H., Mozes, M., and Najarian, J. S. Prolongation of renal xenografts by simultaneous sequestration of preformed antibody, inhibition of complement, coagulation and antibody synthesis. Transplant. Proceed. 3:538, 1971.
7. Perper, R. J., and Najarian, J. S. Experimental renal heterotransplantation. Transplantation 4:377, 1966.
8. Rosenberg, J. C., Hawkins, E., and Rector, F. Mechanisms of immunological injury during antibody-mediated hyperacute rejection of renal heterografts. Transplantation 11:151, 1971.
9. Sherman, H., Klausner, S. and Cook, W. A. Incident dark-field illumination: A new method for microcirculatory study. Angiology 22:295, 1971.
10. Simpson, K. M., Bunch, D. L., Amemiya, H., Boehmig, H. J., Wilson, C. B., Dixon, F. J., Coburg, A. J., Hathaway, W. E., Giles, G. R., and Starzl, T. E. Humoral antibodies and coagulation mechanisms in the accelerated or hyperacute rejection of renal homografts in sensitized canine recipients. Surg. 68:77, 1970.
11. Williams, G. M., Hume, D. M., Hudson, R. P., Jr., Morris, P. J., Kano, K. and Milgrom, F. "Hyperacute" renal-homograft rejection in man. N.E.J.M. 279:611, 1968.

DISCUSSION

Chairman Frank J. Veith: The paper is now open for discussion.

Questioner: (Inaudible)

Dr. Cook: We are in the process now of trying to attenuate the process. We have made the observations just over the last 6 months, and one of our first attempts is to pretreat the animals with steroids. I don't think it's premature to say that pretreatment about 4 hours before either renal or pulmonary xenografts seems to slow down the rate of red cell aggregation and prolongs the entire rejection process, including the edema phase and hemorrhaging. We hope to use this method of attenuation to retard the acute rejection process so that we will be able to describe it more clearly and thoroughly.

Gerald Moss, Rennselaer Polytechnic Institute, Troy, N.Y.: Actually I want to ask something that relates to Dr. Veith's earlier comments regarding our exchange, here, of some specific techniques. In our lab we needed a denervated left lung in a dog with a normal right lung, and we developed a series of procedures so that denervation can now be accomplished in under an hour by technicians, and, most importantly, with never more than ten minutes of ischemia. There are a couple of tricks involved, but the most important is to do the atrial cuff without interrupting blood flow. We do this with a wire loop inside the left atrium, which we secure in place with staples. And the only ischemia is during the transsection and reanastomosis of the pulmonary artery, which we also have techniques to speed up. With only ten

minutes ischemia time, you can have almost 100 per cent long-term survival with xenon studies. The technique can be used practically for large volume experiments.

CHAIRMAN: Thank you very much Dr. Moss. I've seen Dr. Moss' technique, and it does represent quite an advance. Are there any other questions?

J. MOOR-JANKOWSKI, New York University School of Medicine: What were the levels of heteroagglutinins in the serum of the donor animal, which of course would come from the interstitial tissue into the lung after the transplantation?

DR. COOK: Do you mean in the serum of the recipient animal or donor animal? We attempted to measure and wash out all the donor heteroagglutinins. And when we measured agglutination titers on the eluate from the washed out cat lung, we saw no agglutination activity.

DR. MOOR-JANKOWSKI: From the eluates from what?

DR. COOK: The eluates from the perfusion before the lung was hooked into the circuit. It's possible that there were interstitial antibodies we couldn't measure, but in our *in vitro* system for measuring heteroagglutinins we did not detect any, and we made a specific effort to wash them all out.

DR. MOOR-JANKOWSKI: But did you check the level before the transplantation?

DR. COOK: Yes, we checked the level as best we could by washing the organs and measuring the eluate.

DR. MOOR-JANKOWSKI: What I want to know is if you measured the titer and if you knew what it was before?

CHAIRMAN: Yes, we did. In the serum it's extremely high, and it varies from animal to animal.

ACUTE BOVINE PULMONARY EMPHYSEMA: EVALUATION OF TRYPTOPHAN INDUCTION

R. A. Kainer, C. S. Card and G. P. Epling*

Consistent ultrastructural changes occur at the blood-air barrier during the initial stages of the poorly-defined disease complex, acute bovine pulmonary emphysema. Intracellular edema, organellar degeneration and rupture of the plasma membrane in the mebranous pneumonocyte is followed by the same sequence of events in the endothelial cell and, finally, by degeneration and disruption of the basal laminae of these cells. Nine attempts were made to induce this disease complex under controlled conditions in order that pathophysiologic parameters could be correlated with morphologic changes. Eight cows (6 Herefords, 1 Brown Swiss-Jersey crossbred and 1 Holstein-Friesian) varying in age from 5 to 15+ years and varying in physical condition from excellent to poor were used in these trials. Intraruminal administration of 0.7 g d,l-tryptophan per kg of body weight or 0.35 g, 0.525 g or 0.7 g of l-tryptophan per kg of body weight failed to induce clinical signs of acute bovine pulmonary emphysema in any of the cows. Cardiovascular, pulmonary and hematologic parameters remained unchanged with the exception of three instances in which there was a marked but transitory elevation of pulmonary arterial pressure one to four days following the administration of tryptophan. In one of these three cows alveolar septal degeneration and epithelial proliferation were observed in microscopic sections from a diaphragmatic lobe. It was suggested from the ultrastructural cytology of the proliferated cells that they were derived from bronchiolar epithelium.

INTRODUCTION

An animal afflicted with acute bovine pulmonary emphysema is typically a middle-aged, lactating cow which has been moved from a dry, late summer pasture to a lush, green, usually irrigated pasture.[1,2,3] Within a few days she becomes severely dyspneic with her head extended and her breathing characterized by a forced expiratory effort. The course of the disease is rapid, and the mortality is high.

The descriptive term, acute bovine pulmonary emphysema, is considered a misnomer by many, since the progressive lesions are not those established for chronic pulmonary emphysema in man. Synonyms such as cow asthma, panting disease, atypical interstitial pneumonia and pulmonary adenomatosis also are not completely accurate descriptions of this syndrome. Notwithstanding semantics and the vagaries of comparative pathology, different investigators[2-5] have described alveolar expansion, thinning and some rupture of alveolar walls, and focal hemorrhage. There is little or no exudative obstruction, but there is a strong suggestion of bronchiolar constriction. Pulmonary edema is seen commonly, and, as the disease progresses, there is an invasion by macrophages, plasma cells and, occasionally, granular leukocytes. Interstitial fibrosis is not pronounced. However, there is an intra-alveolar proliferation of epithelium which has been described as alveolar epithelium[3] but which may be of bronchiolar origin. Marked interstitial emphysema is a complication seen at necropsy in the distended, uncollapsed lungs.

A SEQUENCE OF ALVEOLAR DESTRUCTION

Epling, who earlier described the ultrastructure of the blood-air barrier in the bovine lung,[6] has elucidated the sequences of ultrastructural change in the thinning alveolar septa.[7] A uniform sequence of degeneration of the alveolar wall was observed in regions where there was some alveolar hemorrhage.

Intracellular edema occurred first in membranous pneumonocytes. There was gradual reduction of micropinocytotic vesicles, enlargement of the cisterns of endoplasmic reticulum, mitochondrial swelling, expansion of the cytoplasmic matrix, and, finally, rupture of the plasma membrane which exposed the alveolar basal lamina to the lumen of the alveolus. This sequence then took place in the same manner within the pulmonary capillary endothelial

* College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, Colorado.

cells. Following destruction of the endothelial cells the basal laminae of the alveolar walls were ruptured, permitting hemorrhage into the alveolar lumen. Thus well-defined morphologic criteria for assessing damage to the blood-air barrier have been established.

RESULTS OF TRYPTOPHAN ADMINISTRATION

Since so-called acute pulmonary emphysema occurs in the ox soon after a drastic change in feed, the possibility of a metabolite from the digestive tract relating to the pulmonary pathology has received attention.[4,8,9,10] Two routes have been suggested: (1) a compound could be absorbed from the stomach or intestine and follow a hematogenous route to the lungs; and (2) a gaseous metabolite may be eructated from the rumen and inhaled.[8] Three to seven times as much eructated ruminal gas follows this route as that which is eructated immediately to the outside.[11] In order to correlate the pathophysiology of acute bovine pulmonary emphysema to morphologic changes, it is essential to establish a reliable experimental method for provoking the disease. Experimental induction of an acute respiratory syndrome resembling bovine pulmonary emphysema by the intraruminal administration of d,l-tryptophan has been reported.[4,9,10] We attempted to induce the syndrome by this method.

Eight cows (6 Herefords, 1 Brown Swiss-Jersey crossbred and 1 Holstein-Friesian) five to over 15 years of age and in varying physical condition were fed a diet of grass hay at least 30 days prior to the intraruminal administration of d,l- or l-tryptophan. The major plants in the grass hay were timothy *(Phleum sp.)*, bluegrass *(Poa sp.)*, redtop *(Agrostis sp.)*, hairgrass *(Deschampsia sp.)*, and a sedge *(Carex sp.)*. Dosages varied from 0.7 g of d,l-tryptophan* to 0.35 g, 0.525 g or 0.7 g of l-tryptophan** per kilogram of body weight. The total mass of tryptophan (150 to 300 g) was suspended in 6 to 8 liters of water, and the suspension was pumped into the rumen through a stomach tube. The cows were permitted continued access to hay and water.

* Nutritional Biochemical Corp., Cleveland, Ohio.
** Sigma Chemical Co., St. Louis, Missouri.

Previous to the administration of the tryptophan, a common carotid artery was exteriorized so that arterial as well as jugular blood samples could be collected for analysis of pH, P_{CO2}, P_{O2}, oxygen saturation and hemoglobin content. The hematologic determinations and pulmonary function tests were performed before tryptophan was given and at intervals following administration. Tidal volumes were recorded utilizing a face mask with an attached pneumotachometer connected to a transducer. An intraesophageal tube with a terminal cuff was used to ascertain transthoracic pressure. A catheter was passed through a jugular vein and the right heart to the pulmonary trunk where pulmonary arterial pressure was relayed by means of a transducer. The transducers were monitored on a Sanborn recorder.

Following the administration of tryptophan, the cows were observed for clinical signs, and hematologic and pulmonary parameters were determined sporadically for from one to five days. Results were essentially negative in that the classic signs of acute bovine pulmonary emphysema were not observed in any of the eight cows used in the experiments. One physiologic parameter, the pulmonary arterial pressure, varied significantly in three cows. A dose of 0.7 g d,l-tryptophan per kg of body weight was given to a six-year-old Hereford cow (A205, Table I). There was no physiologic response. After three weeks a subsequent dose of 0.35 g

TABLE I.—*Intraruminal Administration of Tryptophan to Hereford Cows*

Cow / Dose/Kg Body Weight	Age	Time (hrs)	Pul. Art Pres. (mm Hg)
A205	6 yr	Pretreatment	28
0.7 g d,l-tryptophan		24	25
		48	25
		72	25
		96	24
0.35 g l-tryptophan		Pretreatment	23
		72	50
		96	32
		120	23
A201	6 yr	Pretreatment	20
0.7 g l-tryptophan		48	27
		72	28
		96	50
		120	50
480	15+ yr	Pretreatment	70
0.525 g l-tryptophan		24	115
		72	62

l-tryptophan per kg of body weight was given, and, once again, clinical signs were not apparent. However, her pulmonary arterial pressure doubled 72 hours after treatment and then decreased to normal pressure by 120 hours. She was not slaughtered at that time.

This marked increase in pulmonary arterial pressure was noted in two other cows. In a six-year-old Hereford (A201, Table I) there were no clinical signs following a dose of 0.7 g l-tryptophan per kilogram of body weight, but her pulmonary arterial pressure doubled on the fourth and fifth days after treatment. At postmortem examination following immobilization with succinylcholine chloride gross pulmonary lesions were equivocal. Mild lesions were observed in microscopic sections from the right diaphragmatic lobe. Epithelial proliferation without attendant inflammatory changes was observed at the light microscopic level. Under the electron microscope the proliferated cells resembled bronchiolar epithelial cells more closely than pneumonocytes (Figure 1). In surfaces not covered by these cells degeneration of the blood-air barrier was observed (Figure 2).

A very old, weak Hereford cow (480, Table I) had a hypertensive pulmonary arterial pressure (70 mm Hg) prior to treatment. It increased 1.6 times (to 115 mm Hg) 24 hours following the administration of 0.525 g l-tryptophan per kilogram of body weight. She was down in right lateral recumbency in the corral on the second day, and her pulmonary arterial pressure was not recorded. On the third day her pulmonary arterial pressure was slightly below the pretreatment level. At postmortem examination following euthanasia there was chronic suppurative pneumonia in the ventral one-third of all lobes of the left lung with strong adhesions to the parietal pleura. The gross appearance of the right lung was normal. Significant pulmonary lesions were lacking in microscopic sections from this lung.

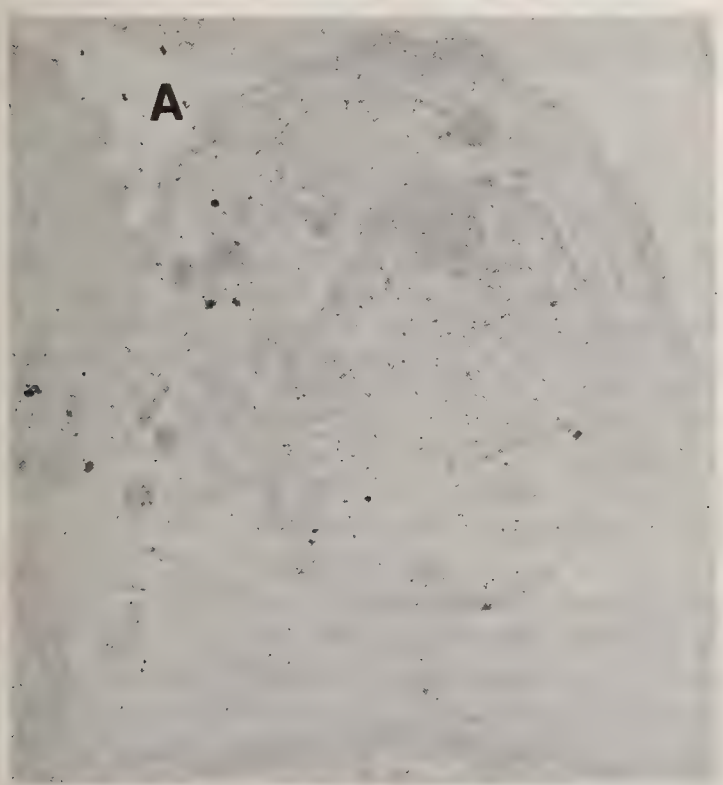
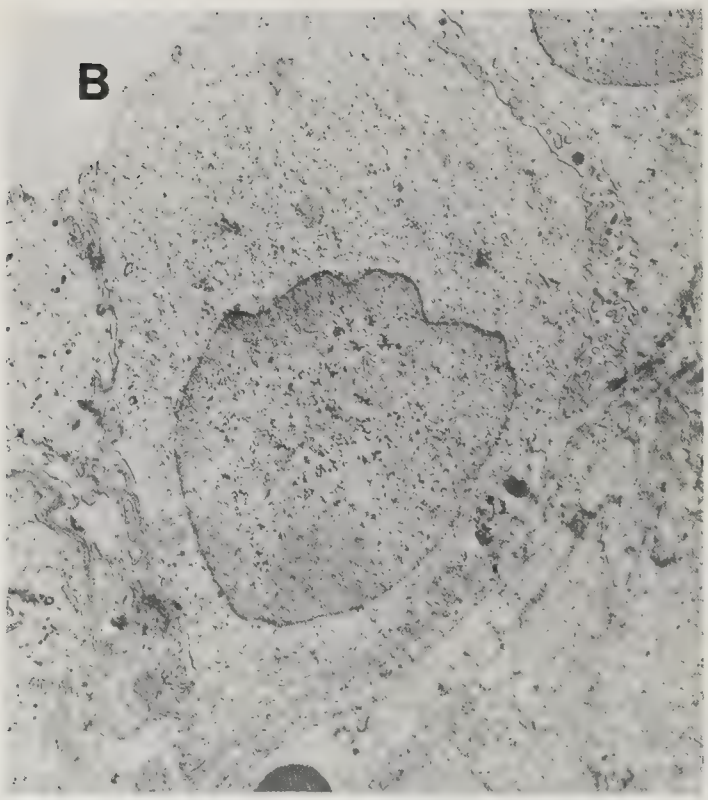


FIGURE 1.—Electron micrograph of a proliferated cell lining an alveolus. Orig. mag. 4300×.

DISCUSSION

In our hands the intraruminal administration of d,l- or l-tryptophan did not induce clinical signs resembling those ascribed to acute bovine pulmonary emphysema. Johnson and Dyer[9] reported that five out of eight cattle died following the oral administration of d,l-tryptophan. Dickinson et al.[4] described clinical signs of respiratory distress and pulmonary lesions varying from mild to severe in seven to eight cows receiving oral d,l-tryptophan. Seven cows given oral doses of this amino acid by Carlson et al.[10] developed typical signs of interstitial pulmonary emphysema. Four of these cows died in 1½ to 4½ days following tryptophan administration. The conditions of our experiments were based on those described in the papers cited above.[4,9,10]

One can speculate on nutritive variations in the grass hay fed in these geographically separated experiments; there are no definite data on such differences. Three old cows in poor physical condition were selected on the premise that they would be more likely to have had previous pulmonary insults. Even with this experimental bias, clinically observable signs of acute pulmonary emphysema could not be provoked by the administration of tryptophan. If previous pulmonary pathology were invariably predisposing, then the old, debilitated Hereford cow with chronic suppurative pneumonia should have responded with clinical signs of acute res-



Electron micrograph of a bronchiolar epithelial cell. Orig. mag. 6400×.

piratory distress. She did not respond in this manner.

Among the several parameters measured only the pulmonary arterial blood pressure varied significantly in three cows. In one of these three, alveolar septal degeneration and epithelial proliferation may have been related to the administration of l-tryptophan, or the condition may have preexisted. This cow was clinically refractive.

The precise etiology and pathogenesis of this ill-defined syndrome have yet to be elucidated.

SUMMARY

Eight cows were given intraruminal doses of d,l- and/or l-tryptophan. None of the cows developed clinical signs of acute bovine pulmonary emphysema. Among the cardiovascular, pulmonary and hematologic parameters measured on these animals the pulmonary arterial blood pressure increased significantly in three cows.

In one of the three cows with increased pulmonary arterial pressure, alveolar septal degeneration and epithelial proliferation were observed in microscopic sections from a diaphragmatic lobe. Ultrastructural studies suggested that the proliferated cells were derived from bronchiolar epithelium.

ACKNOWLEDGEMENTS

The technical assistance of R. C. Borgman, F. D. Giddings and A. E. Kilminster is gratefully acknowledged.

REFERENCES

1. Tucker, J. O., and Maki, L. R. Acute pulmonary emphysema of cattle. I. Experimental production. Am. J. Vet. Res. 23:821–823, 1962.
2. Gibbons, W. J. Bovine pulmonary emphysema. Mod. Vet. Prac. 43:34–38, 1962.
3. Moulton, J. E., Harrold, J. B., and Horning, M. A. Pulmonary emphysema in cattle. J.A.V.M.A. 139:669–677, 1961.
4. Dickinson, E. O., Spencer, G. R., and Gorham, J. R. Experimental induction of an acute respiratory syndrome in cattle resembling bovine pulmonary emphysema. Vet. Rec. 80:487–489, 1967.
5. Dickinson, E. O. The bovine pulmonary emphysema complex. Bull. Path. 73, 1967.
6. Epling, G. P. Electron microscopy of the bovine lung: The normal blood-air barrier. Am. J. Vet. Res. 25:679–689, 1964.
7. Epling, G. P., and Wilson, J. W. Uniformity of sequences in pulmonary alveolar necrosis. Am. J. Vet. Res. 32:471–477, 1971.
8. Moulton, J. E., Cornelius, C. E., and Osburn, B. I. Acute pulmonary emphysema in cattle. J.A.V.M.A. 139:669–677, 1963.
9. Johnson, R. J., and Dyer, I. A. Effect of orally administered tryptophan on tryptophan pyrrolase activity in ovine and bovine. Life Sci. 5:1121–1124, 1966.
10. Carlson, J. R., Dyer, I. A., and Johnson, R. J. Tryptophan-induced interstitial pulmonary emphysema in cattle. Am. J. Vet. Res. 29:1983–1989, 1968.
11. Dougherty, R. W., and Cook, H. M. Routes of eructated gas expulsion in cattle—a quantitative study. Am. J. Vet. Res. 23:997–1000, 1962.

DISCUSSION

Aran Johnson, University of Michigan, Detroit: I'd like to know what steps you took to assure that the intravenous catheter was indeed placed in the pulmonary artery rather than, say going down in another vessel, or for that matter just looping around in the right ventricle and coming back into the atrium.



FIGURE 2.—Electron micrograph of degeneration of the alveolar epithelium in a part of an alveolus not covered by proliferating cells. Arrows indicate basal lamina. Orig. mag. 4300×.

DR. KAINER: This is the one parameter I feel secure about. Dr. Alexander is going to talk about it. Actually, you can follow the catheter through the right heart very nicely. You can tell when you're in the right atrium. You can tell when you're in the ventricle, and you can tell when you go through the valve into the pulmonary artery.

DR. JOHNSON: Is this by pressure changes that you're measuring?

DR. KAINER: By pressure changes on the monitor. It's very simple.

B. C. HOSTZIOLOS, Maryland University, College Park, Md.: Have you any experience with urea poisoning or parathion poisoning in cattle?

DR. KAINER: Yes, I did work years ago with some urea poisoning. You've brought up a good point: where do we go from here? What other metabolites do we investigate? We're thinking of terpenes. Dr. Don Olsen, who's currently conducting work at the Medical School at the University of Colorado, is concerned with this.

H. A. LYONS, Downstate Medical Center, Brooklyn, N.Y.: Did you ever think about analyzing the blood supply after you gave the tryptophan?

DR. KAINER: This has been done by the workers at Washington State.

DR. LYONS: Because that would be crucial to your experiment.

DR. KAINER: Yes, They found no essential variation. They could not provoke the disease in cattle or sheep by intravenous or interperitoneal means.

CHAIRMAN LENFANT: Excuse me, if I may elaborate a bit further, Dr. Lyons. I don't think that's a real issue. The real issue is to measure how much tryptophan goes into the pulmonary artery and how much comes out. It seems to me

that for the type of lesion he's observing, what is needed, at this time, is to know what happened to the tryptophan in the lung and whether it has been metabolized or not.

DR. KAINER: Dr. Dickinson at Wahington State is working on this. He's looking for serotonin. We have a little sleeper here. I left it until now. After three, six, twelve, and twenty-four hours, we found an increase in tryptophan in the urine. I haven't done enough to report on it yet.

BURLING, Howard University: I was interested in what you had to say about terpenes. I published previously on turpentine toxicity. When it's given orally, but not by inhalation, there is a great deal of lung damage.

J. R. GILLESPIE, University of California, Davis: I'm curious as to what the vascular response is in these animals as compared to the high altitude bovine disease, brisket disease. In other words, I've always been sort of struck by the possibility that the initial insult may be very similar—severe pulmonary hypertension. In that case, it would be interesting to compare your high altitude cattle with low altitude cattle in Washington.

DR. KAINER: This is a good thought. Dr. Card, who is on the project and was concerned mainly with this aspect of it, has considered this. Of course, we are ambient there at Fort Collins, at a little over 5,000 feet. Now, some of the cows might have been at six or seven thousand. Up on the high plains, it's easy to reach 10,000 feet in altitude. We have entertained the idea of putting a cow in the chamber and really taking her up.

J. C. NORMAN, Boston City College, Massachusetts: This may be pure naivete, but could I ask you how you feel this works? Has there been any labeled tryptophan given? Does it elicit a positive inotropic response? Does it constrict pulmonary capillaries? Does it increase the mechanism of the right ventricle? I have no ideas, and I'm not familiar with the area. Could you help me?

DR. KAINER: I'm pleased that you have the question. We're thinking about it, and so is Dr. Dickinson's group up at Washington State. Perhaps a metabolite of tryptophan is acting on the smooth musculature of the bronchioles and/or the vessels. Serotonin of course has tryptophan as its precursor molecule.

CHRONIC OBSTRUCTIVE LUNG DISEASE IN HORSES

J. R. Gillespie and W. S. Tyler*

Tyler and co-workers showed the morphology of the lungs of the horse resembled those of man more closely than those of dogs, cats, swine, monkeys (Rhesus), cattle, sheep, rabbits, rats or guinea pigs. This group confirmed earlier studies that showed the horse had naturally occurring pulmonary emphysema and chronic bronchitis which were similar to these diseases in man. The incidence of these diseases in the horse population is not known. Certain feed and environmental conditions have historically been reported as being important in these diseases.

We found horses with chronic obstructive lung disease have greater than normal pulmonary arterial pressure and less than normal lung compliance and pulmonary diffusion capacity. These animals have an increased pulmonary resistance and initial studies indicate an impairment to maximum expiratory flow rate at various lung volumes.

Quantitative electron microscopic studies have shown a decrease in pulmonary capillaries/cm of alveolar wall as well as other cell population changes in the emphysematous horse lung.

Most recent studies of scanning electron micrographs of sections of emphysematous horse lungs have shown new "scale-like" lesions heretofore not seen in emphysematous lungs.

INTRODUCTION

We reviewed the comparative research on chronic respiratory disease in the horse in 1968.[1,2] Herein we intend to update that review.

The role of certain phenomena that are common to all living tissue versus special phenomena that are peculiar to individual species pinpoints the importance of animal selection for experimental studies. We postulated that structural arrangements of lungs are important in determining their susceptibility to certain diseases. We took note that horses were reported to have chronic bronchitis and alveolar emphysema in a form similar to that which occurs in man.[3,4] We confirmed earlier studies that showed the similarity of these diseases in horses and human beings.[1]

* University of California, Davis.

DISCUSSION

Tyler and co-workers [5] studied gross and subgross anatomical features of lungs from nine mammal species. They concluded that of these species, the horses had a lung most like that of man. A summary of their results is shown in Table I.

We examined the ultrastructure of the normal and emphysematous lungs of more than sixty horses with the conventional electron microscope. The general morphology of the blood/air pathway and the individual cell types were found to be similar to those of other species.[6,7] We determined the pulmonary capillary and cell density of normal and emphysematous lungs

TABLE I.—*Subgross Morphology of Horse Lung Compared with Seven Other Species*

Species	Lobules	Pleura	Typical Distal Airway	Structures Supplied by the Bronchial Artery
Horse Man	Incompletely separated Extensive interlobular connective tissue	Thick	Terminal bronchiole Respiratory bronchioles are rare and poorly developed	Bronchi, vasa vasorum of PA & PV Pleura and interalveolar tissue Some interalveolar septa
Cattle Sheep Swine	Completely separated Extensive interlobular connective tissue	Thick	Terminal bronchiole Respiratory bronchioles are rare	Bronchi, vasa vasorum of PA & PV Pleura and interlobular tissue
Dogs Cats R. Monkey	Very poorly defined Little interlobular connective tissue	Thin	Respiratory bronchiole Terminal bronchioles are short	Bronchi, vasa vasorum of PA & PV

TABLE II.—*Number of Vascular Components per Centimeter of Interalveolar Septum as Seen by Electron Microscopy in Normal and Emphysematous Horses*

	Normal			Emphysematous		
	Capillaries	Endothelial Nuclei	RBC	Capillaries	Endothelial Nuclei	RBC
Mean	252.4	87.4	384.4	61.8	57.3	242.6
Sx [a]	25.0	3.68	33.7	13.3	4.7	29.0
P< [b]	0.01	0.001	0.05			

[a] Deviation from mean. [b] Probability.

TABLE III.—*Blood-Gas Tension and pH Values at Rest and Immediately Following Exercise* [a]

	Pa_{O_2} (mm Hg)		Pa_{CO_2} (mm Hg)		pHa	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Horses Free of Respiratory Disease [9]						
X	103	96	43	43	7.47	7.48
Sx	±4.9	±4.8	±3.6	±5.2	±.006	±.016
Horse with Respiratory Disease [9]						
X	80	69	46	49	7.45	7.39
Sx	±5.5	±6.4	±12.1	±17.8	±.016	±.035
P<	0.01	0.01	NS [b]	NS [b]	NS [b]	0.05

[a] x, mean; Sx, deviation from mean; P, probability.
[b] Not statistically different from control group.

with quantitative electron microscope techniques.[8] The results are shown in Table II. The apparent loss of capillaries in the emphysematous lungs correlates well with the lower pulmonary diffusion capacity and hypoxemia in emphysematous horses (Tables III–V).

More recently, Leith and Gillespie have examined the flow-volume and flow pressure characteristics of the lungs of these horses.[9] Average values for six normals and values for a single horse with emphysema are shown in Table VI.

Maximum expiratory flows at FRC were 65–90 L/sec in four normals and only 2 L/sec in the horse with emphysema. Figure 1 compares the volume-flow trace of a normal horse with that of the horse with emphysema (COLD).

Our pulmonary function measurements show the horse to have proportional values to those in man and that there is similar loss in function when they are afflicted with emphysema. The more difficult task is the correlation of the loss of function with structural changes. New quantitative techniques to evaluate the lung struc-

TABLE IV.—*Pulmonary Vascular Shunt ($\dot{Q}s/\dot{Q}t$) in Normal and Emphysematous Horses*

	$\dot{Q}s/\dot{Q}t$ (%) (ml/min/mm Hg)	
	Normal	Emphysematous
Mean (%)	5.24	8.90
Sx [a]	0.74	1.21
p< [b]	0.02	—

[a] Deviation from mean.
[b] Probability.

TABLE V.—*Pulmonary Diffusing Capacity for Carbon Monoxide ($D_{L_{CO}}$) of Normal and Emphysematous Horses*

	$D_{L_{CO}}$	
	Normal	Emphasematous
Mean	365.9	236.4
Sx [a]	31.3	17.5
P< [b]	0.01	—

[a] Deviation from mean.
[b] Probability.

TABLE VI.—*Respiratory Mechanics of Normal Horses and One with Emphysema*

	Normal Horses [6]	Emphysematous Horse
Body Weight (Kg)	452	363
Lung Weight (Kg)	4.1	5.9
FRC (L)	18.9	37.8
RV (L)	9.1	16.1
TLC (L)	42.1	58.6
C_L (L/cmH_2O)	3.4	4.2
C_{cw} (L/cmH_2O)	0.9	0.9
R_L (cmH_2O/L/sec)	0.2	0.15
R_{cw} (cmH_2O/L/sec)	0.16	0.10
R_{RS} (cmH_2O/L/sec)	0.40	0.27

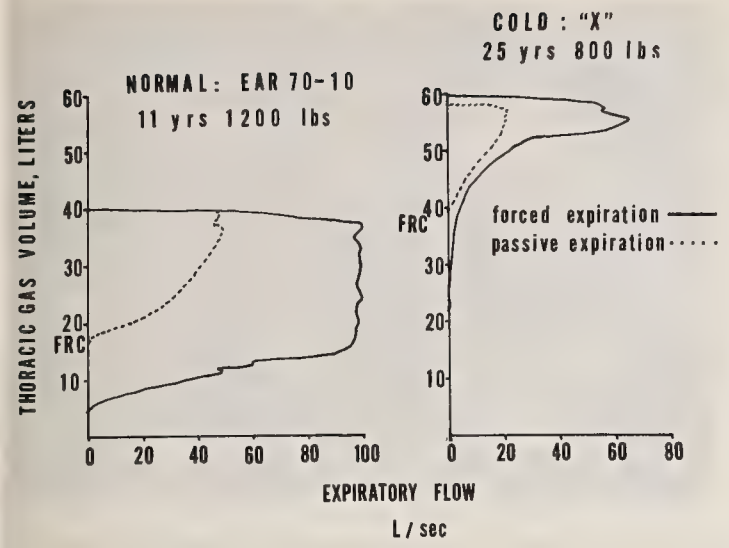


FIGURE 1.—Thoracic gas volume—flow curves of a normal horse (left) and horse with chronic obstructive lung disease (COLD) (right). Broken lines are passive expirations from total lung capacity.

ture will be important to our complete understanding of the mechanisms underlying the loss of lung function.

Nowell and co-workers have developed new ways of examining the lung with the scanning electron microscope (SEM).[10] Figure 2 is a SEM micrograph of a section of normal horse lung. The principal advantages of the SEM are its great depth of focus and wide range of magnification which, in the lung, permit large numbers of alveoli to be seen in focus at one time. It is especially useful for studies of emphysema, due to the focal nature of the lesions and

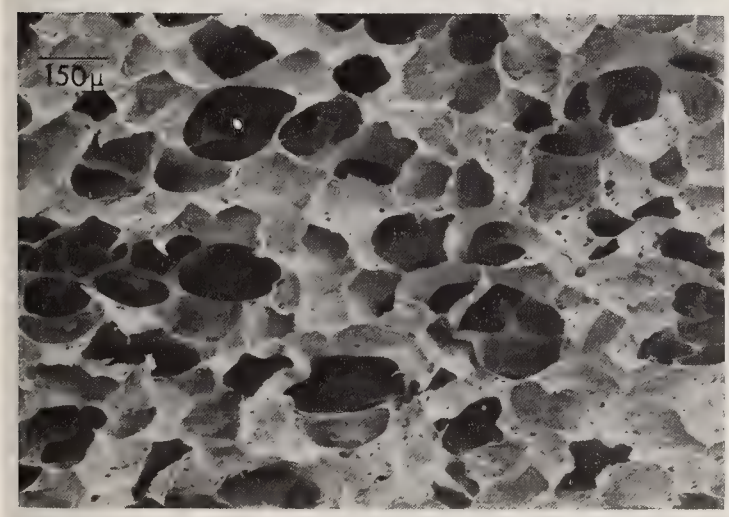


FIGURE 2.—SEM of normal horse lung. Normal sized alveoli and alveolar ducts.

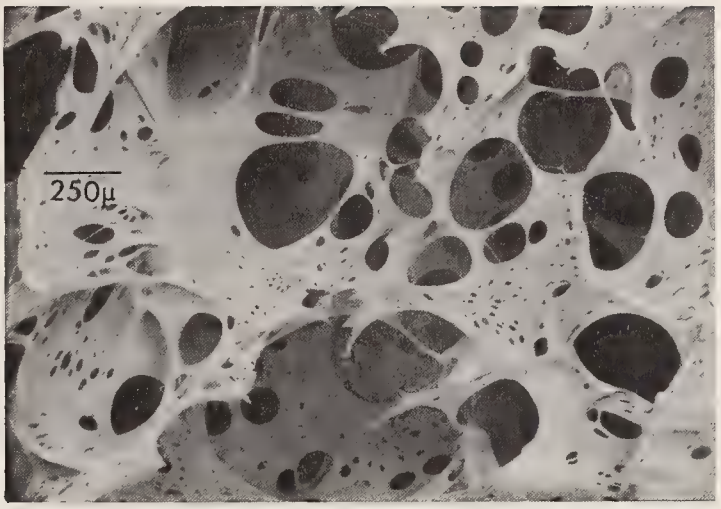


FIGURE 3.—SEM of emphysematous horse lung. The alveolar walls are fenestrated and sparce. Most interalveolar septa have been completely destroyed, while trabecular strands of tissue are the sole remnants of others (1 - O'clock).

the variation in the amount of destruction of adjacent areas.

Figures 3 and 4 show emphysematous horse lung. Fenestrae in the walls of alveoli, alveolar ducts, and respiratory bronchioles are recognized as the earliest and most characteristic manifestation of emphysema in man.[11] Isolated pieces of tissue apparently floating in alveolar spaces are a pathognomonic lesion of emphysema in histological sections.[11] This appearance is the result of seeing trabeculae in a single plane.

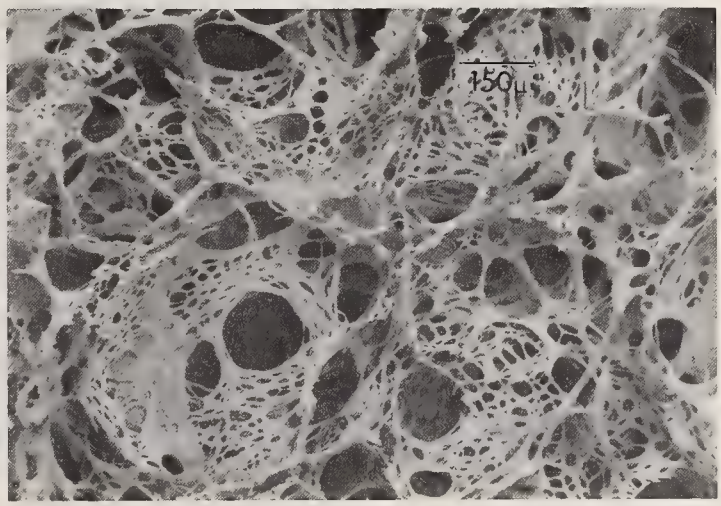


FIGURE 4.—SEM of emphysematous horse lung. Remnants of a destroyed respiratory bronchiole. Only vague skeleton of normal lung parenchyma remain.

More isolated pieces of tissue are seen in thin, i.e., 1–4 u, sections of emphysematous lung than in thick, i.e., 100 u sections, both of which are commonly examined with a light microscope. The isolated pieces are not seen in the 2/mm slices used for sub-gross studies, or in SEM micrographs, because at least one end of most trabeculae are attached to the thick specimens (Figure 5).

We saw scale-like plaques only in SEM micrographs of emphysematous lung lesions (Figures 5, 6 and 7). These have heretofore not been described. Whereas we have had excellent correlation between cell types observed by transmission and SEM in studies of normal lung, we are to date unable to completely characterize the plaques. They have some of the characteristics of alveolar macrophages; it is also possible that they could represent desquamated type I or type II alveolar epithelial cells. Neither of these possibilities seem likely as we have not seen plaques in normal areas of the emphysematous horse lungs, in lungs from normal horses, or in lungs of other species of mammals. We are now examining emphysematous lung tissue from human beings to determine if plaques are present.

SUMMARY

Our studies in horses have shown that chronic obstructive lung disease has resulted in greater than normal pulmonary arterial pressure and less than normal lung compliance and pulmonary diffusion capacity in the animals. The increased pulmonary resistance and impaired maximum expiratory flow rate at various lung volumes were characteristic of these animals as defined in initial studies. The electron microscopic studies have quantitatively shown decrease in pulmonary capillaries per cm of alveolar wall as well as other cell population changes in the emphysematous horse lung. Also, as indicated above, the most recent studies involving scanning electron micrographs of sections of emphysematous horse lungs have shown new "scale-like" visions not previously seen in the emphysematous lungs.



FIGURE 6.—SEM of emphysematous horse lung. Plaque on the junction of anastomosing trabeculae.



FIGURE 5.—SEM of emphysematous horse lung. Cut edge of trabecula and interalveolar septum. A plaque is at the junction of the trabecula with the septa.

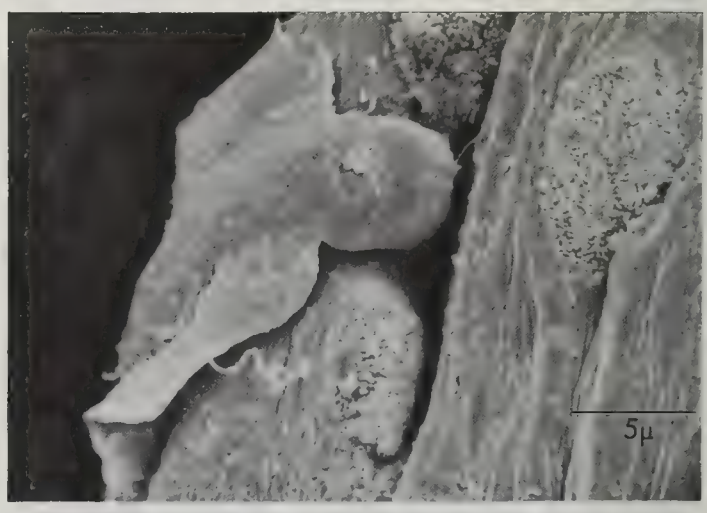


FIGURE 7.—SEM of emphysematous horse lung. Plaque and type II alveolar epithelial cell on surface of trabeculae.

ACKNOWLEDGMENT

We wish to thank Miss J. A. Nowell, Department of Anatomy, University of California, Davis, for the scanning electron micrographs. These studies were supported in hand by NIH grants ES AP 00628 and FR 06138.

REFERENCES

1. Gillespie, J. R. and Tyler, W. S. Chronic alveolar emphysema in the horse. Adv. Vet. Sci. Comp. Med. 13:59–99, 1969.
2. Tyler, W. S. and Gillespie, J. R. Structural and functional alterations in horses with emphysema. Proc. Symp. Animal Models for Biomedical Research II, Nat. Acad. Sci., 1969.
3. Stoomer, O., Uber das chronische vesiculare Emphysema namentlich der Pferde lunge. Deutsche Zeitschrift furr Thiermedizin und Vergleichende Pathologie. 13:97–127, 1887.
4. Foley, F. D. and Lowell, F. C. Equine centrolobular emphysema with further observations on the pathology of heaves. Am. Rev. Resp. Dis. 93:17–21, 1966.
5. Tyler, W. S., Gillespie, J. R. and Nowell, J. A. Symposium on pulmonary and cardiac function, I. modern function morphology of the equine lung. Eg. Vet. J. 3:1–11, 1970.
6. Weibel, E. R. Airways and respiratory surface in the lung. Eds. A. A. Liebow and D. E. Smith. Williams and Wilkins Co., Baltimore, p. 5, 1968.
7. Gillespie, J. R. and Tyler, W. S. Quantitative electron microscopy of the interalveolar septa of horse lung. Am. Rev. Resp. Dis. 95:477–483, 1967.
8. Gillespie, J. R. and Tyler, W. S. Capillary and cellular changes in alveolar walls of emphysematous horse lungs. Am. Rev. Resp. Dis. 95:484–489, 1967.
9. Leith, D. E. and Gillespie, J. R. Respiratory mechanics of normal horses and one with chronic obstructive lung disease. (Abstract) Fed. Proc., April, 1971.
10. Nowell, J. A., Gillespie, J. R. and Tyler, W. S. Scanning electron microscopy of chronic pulmonary emphysema: A study of the equine model. (Abstract) Proc. 4th SEM Symp. IIT Res. Inst., Chicago, April, 1971.
11. Pratt, P. C. and Kilburn, K. H. A modern concept of the emphysemas based on correlation of structure and function. Human Path. 1:443–463, 1970.

DISCUSSION

H. Calderwood, University of Florida, Gainesville: I'd like to know your opinion on the role that allergic alveolitis might play in the clinical picture of heaves in the horse.

Dr. Gillespie: Well, I don't really believe it has been shown that there's a marked alveolitis in these animals. For example, if you compare the reaction in the horse having bronchiolitis and the sheep having frank alveolitis, the response is quite different. In other words, you do not see the hypertrophy of the alveolar lining cells, particularly the type II cells, in the horse as you do in the sheep. So, the correlation between those two diseases is not good. I have never seen a reaction that I felt was an inflammatory alveolitis in a horse with heaves, or with emphysema, or with bronchiolitis.

Dr. Calderwood: I'd also like to know how you do your maximum forced expiratory rates in the horses.

Dr. Gillespie: These horses are suspended in a body sling and anesthetized. Through a tracheal cannula, the animal's lung is inflated to total lung capacity, or 30 cm of water. He then expires into a bank of evacuated drums.

Sung S. Park, Yeshiva University, Bronx, N.Y.: The forced expiratory flow rate at FRC is reduced in the horse with emphysema. I guess the cause of this flow limitation could be related to a narrowing of small airways, or perhaps the collapsability of the large airways. Can you comment on this?

Dr. Gillespie: I think your surmise is correct as far as what is the limiting factor in the maximum expiratory flow rate. We found that there's a marked deviation or variation in the amount of membranous tissue in the trachea of even normal horses, and so it's not terribly clear to us yet as to where the most important limitation is. The hypothesis is that the limiting point along the airways moves toward the alveoli. There's a greater or increasing loss of elasticity of the lung, but we've not established where the equal pressure point is or where the closing point is along the airway.

Dr. Park: What is the incidence of bronchitis in the horse with emphysema?

Dr. Gillespie: We find that in the horse with emphysema there may or may not be bronchitis, or there may or may not be evidence of bronchiolitis. The two diseases do co-exist very frequently, but we have often seen emphysema without evidence of active bronchitis or bronchiolitis.

PATHOPHYSIOLOGIC ASSESSMENT OF A CANINE MODEL OF INDUCED ACUTE RESPIRATORY INSUFFICIENCY

Robert H. Liss and John C. Norman*

Infusions associated with surgical procedures may lead to expansion of intravascular and interstitial fluid volumes. When pulmonary edema and congestion ensue acute respiratory insufficiency (ARI) may result. To assess the magnitude of fluid overload necessary for ARI production, 20 mongrel dogs were infused with normal saline: I) untreated controls; II) intermittently, 50 ml/kg/hr; III) continuously, 100 ml/kg/hr; IV) continuously, 100 ml/kg/hr with furosemide (0.8 mg/kg) every two hours. Scanning/transmission electron microscopy, light microscopic and physiologic assessments were made of pulmonary tissue. Ultrastructural and pathophysiologic correlates of ARI were seen only in group III (continually loaded, no furosemide). Scattered areas of pulmonary pathology included edema i.e., intra-alveolar, granular precipitate and widening of alveolar septa. Congestive changes were dilated alveolar capillaries, distended intra- and inter-membranous spaces, basement membrane spreading, hypertrophic and cuboidal epithelial cells, capillary rhexis and alveolar hemorrhage, RBC's and hemosiderin-filled macrophages in alveolar spaces. Pulmonary tissue ultrastructure and pathophysiologic data from groups I, II and IV were within normal limits. The combined data indicate that massive, continuous fluid overload (100 ml/kg/hr for 4–6 hrs) is required to produce ARI in normal dogs; associated cytopathology is essentially indistinguishable from that observed in "shock lung."

INTRODUCTION**

Acute respiratory insufficiency (ARI) has become an increasingly frequent cause of death following trauma, shock or sepsis.[1] Patients may survive initial hemodynamic insults only to develop a progressive, irreversible pulmonary insufficiency. The importance of recognizing the early pathophysiologic events in the genesis of respiratory failure is reflected in the number of model systems designed to study its development. Experimental acute respiratory and hemodynamic changes have been investigated after trauma and post-surgical shock,[2,3] hemorrhagic shock,[4,5] septic shock,[6] injection of free fatty acids,[7] and pump-oxygenator procedures.[8] Each of these models has been useful in elucidating the sequence of events in the experimental production of pulmonary insufficiency. However, in the last two decades, few studies have been initiated to investigate the role of fluid overload in the genesis of ARI.[9–12]

In order to assess the magnitude and pathophysiologic significance of fluid overload in the production of acute respiratory insufficiency, an investigation of the effects of continuous fluid infusion on pulmonary function and structure has been undertaken.[13] This report correlates the pathologic observations with an extended series of measured and derived respiratory and hemodynamic data.

MATERIALS AND METHODS

Normal mongrel dogs weighing 26 ± 1 (SEM) kg, were anesthetized with sodium thiamylal (Surital, Parke Davis). Fluid overload was effected by infusing lactated Ringer's solution; the experimental protocol is summarized in Table I.

Parameters of respiratory mechanics and the work of respiration were calculated from data on inspiratory and expiratory air flow and transpulmonary pressure. A Respiratory Analog Computer (Division of Experimental Surgery, Naval Medical Research Institute) was used to calculate and display tidal volume and work of breathing for each breath. Lung compliance was calculated by dividing inspired volume by the difference in transpulmonary pressure at each inspiration and each expiration. Transpulmonary pressure was measured as the differential of tracheal air pressure and intraesophogeal pressure. Other relevant phys-

* Department of Surgery, Harvard Medical School, Boston, and Arthur D. Little, Inc., Cambridge, Massachusetts.

** Supported, in part, by The John A. Hardford Foundation, The John and Mary R. Markle Foundation, The Charles E. Merrill Trust, and U.S.P.H.S. Grant No. HE 14294.

TABLE I.—*Experimental Protocol*

Group	Number of Dogs	Experimental Procedure			
		Infusion	Rate	Duration	Intervention
I	3			Untreated Controls	
II	6	Intermittent	50 ml/kg/hr	25 min ea hr for 6 hrs	
III	6	Continual	100 ml/kg/hr	4–6 hrs	
IV	5	Continual	100 ml/kg/hr	4–6 hrs	furosemide (Lasix (furosemide), Hoechst) (0.8 mg/kg) every 2 hrs

iologic parameters monitored included total and resistive respiratory work, hematocrit, urine volume, urine and serum osmolalities, arterial pH and blood gases, EKG, aortic and central venous pressure. The experimental design for collecting these data are presented in Figure 1.

Biopsies of each of the lobes of both lungs were obtained for microscopic examination. For light microscopy, samples of lung were fixed in glutaraldehyde or formalin. Paraffin sections were prepared in the standard manner. Slides were stained with hematoxylin-eosin, phosphotungstic acid-hematoxylin, and Mallory's analine blue.

For transmission electron microscopy, the lung tissue was placed in cold (4°C.) 3% glutaraldehyde in 0.1 M Sorenson's phosphate buffer (pH 7.4) and cut into millimeter-square cubes. The tissue was fixed at 4°C. in glutaraldehyde solution for 24 hours, washed in 0.1 M Sorenson's phosphate buffer, post-fixed in 2% osmium tetroxide in Sorenson's phosphate buffer, rinsed twice in cold distilled water, and dehydrated through a graded series of ethyl

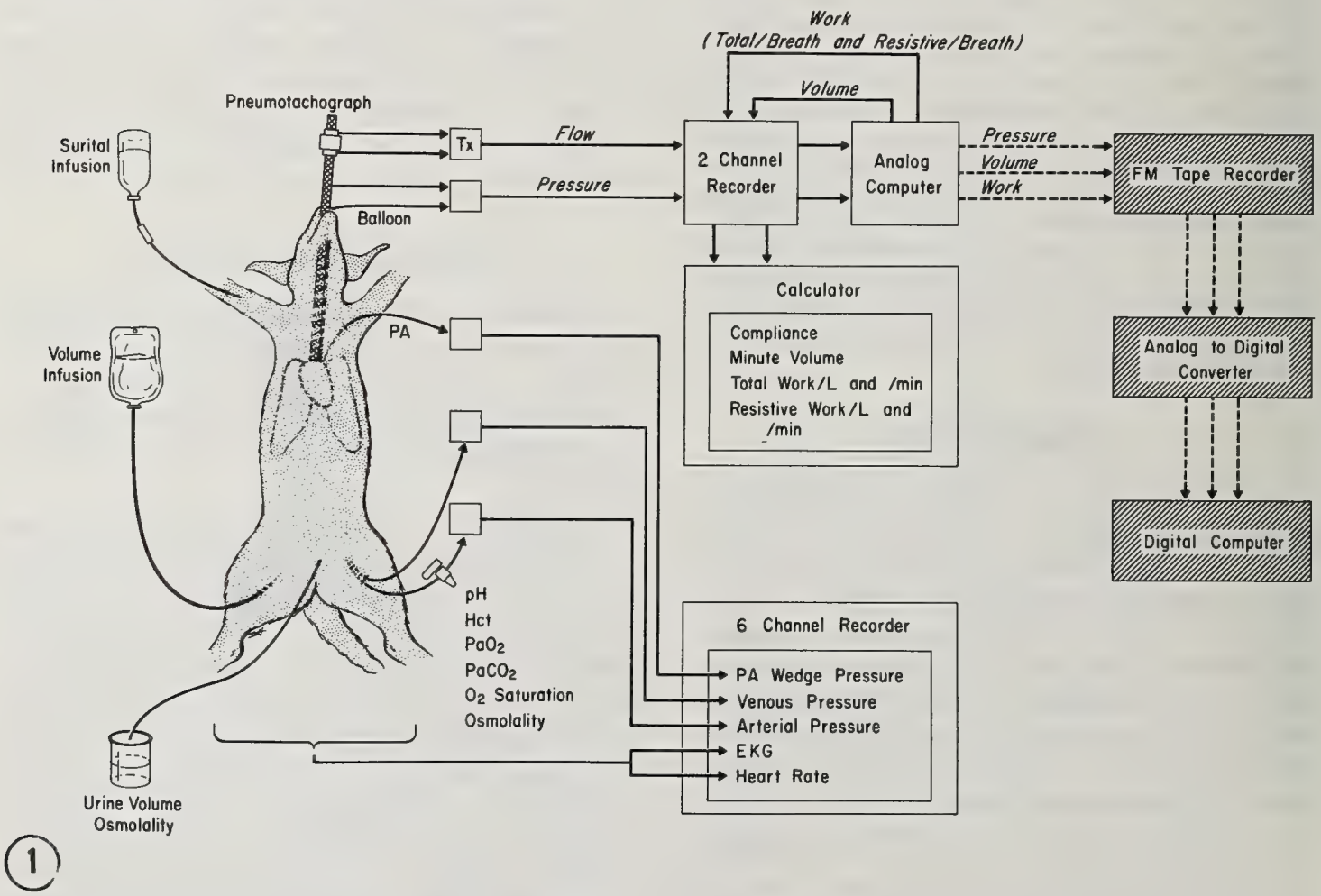


FIGURE 1.—Experimental Design.

alcohols and propylene oxide. Thereafter, the samples were embedded in Epon 812. Tissue sections were picked up on carbon-coated grids and stained with uranyl acetate and lead citrate. The sections were examined in an RCA EMU 3G electron microscope.

Other samples of lung tissue were prepared for scanning microscopy. After overnight fixation in 3% glutaraldehyde millimeter cube samples were rinsed thoroughly in several changes of distilled water. These were transferred to a glass slide and dried at room temperature overnight. Each sample was mounted on an individual specimen holder and transferred to a vacuum evaporator in which the samples were coated with gold. Thereafter the tissue was examined in a Cambridge Stereoscan Electron Microscope (Mark II).

RESULTS

Only animals in Group III showed clinical manifestations of pulmonary edema terminally and died after 5–6 hrs of infusion.

Light Microscopy

Scattered areas of induced pulmonary pathology were observed in Group III dogs; i.e., continually overloaded with lactated Ringer's solution at 100 ml/kg/hr and without furosemide treatment. Lung tissue from Group III dogs was edematous and congested. In Figure 2, interstitial edema, distended capillaries and intra-alveolar hemorrhage are seen. Ingestion of intra-alveolar erythrocytes by alveolar macrophages was observed; the number of clear cytoplasmic vacuoles in these

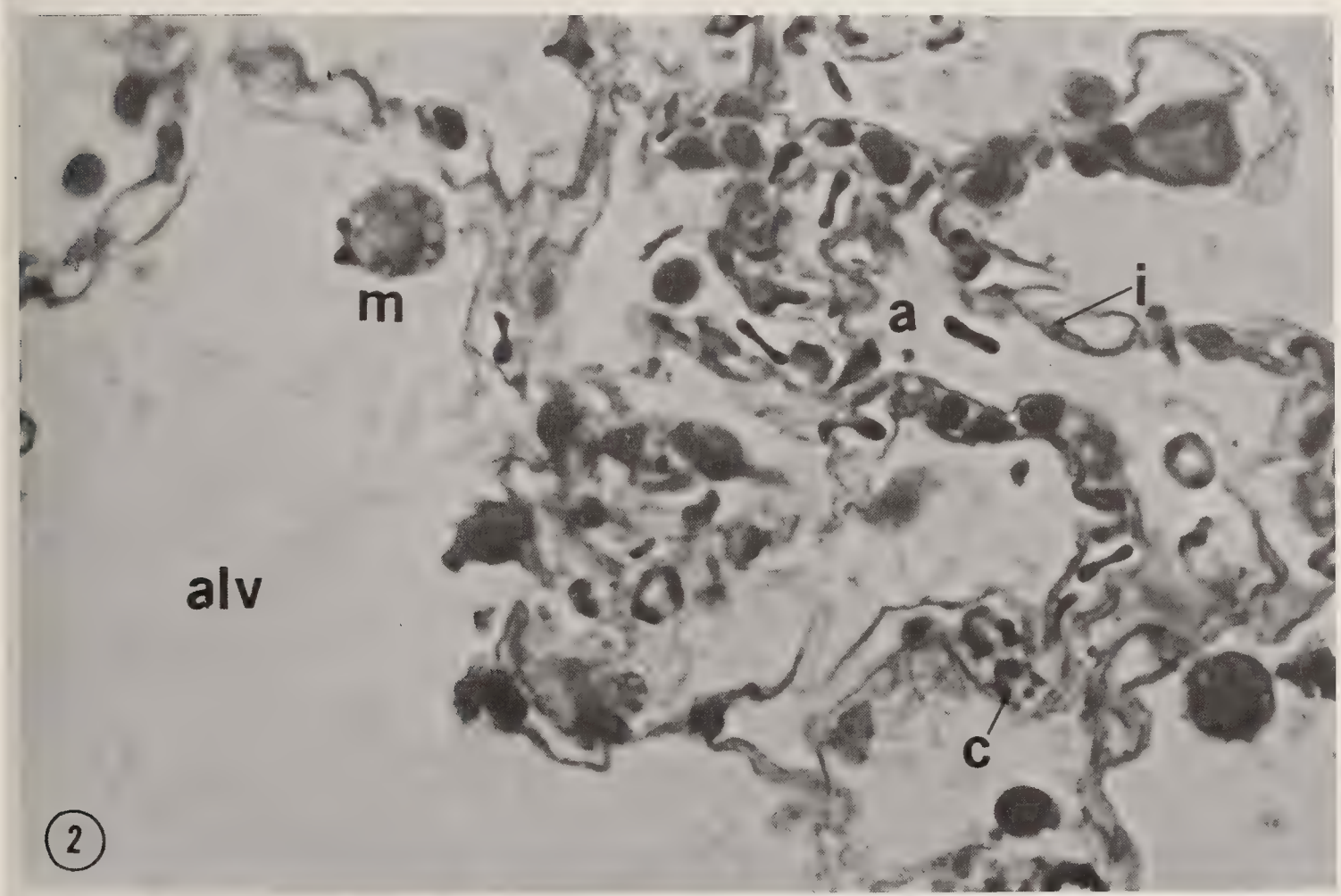


FIGURE 2.—Light photomicrograph of lung tissue from group III (continuously overloaded) dog.

Interstitial edema (i), erythrocytes in alveolus (a) and crenated red blood cells (c) are seen. Note phagocytosis of red cell by macrophage (m) in alveolus (alv). ×600.

macrophages presumably reflected the incorporation of edema fluid. A variety of crenated red cell profiles was observed in the pulmonary capillaries.

Transmission Electron Microscopy

Edema was evidenced by distended interstitial spaces. Collagen fibers were displaced by homogenous areas corresponding to accumulated edema fluid. Thickened basement membranes and enlarged irregular plasmalemmae characteristically defined the areas of intercellular accumulation of fluid. Intense pinocytotic activity among both epithelial and endothelial cells was evident; additionally, some epithelial cells were extensively vacuolated. Red blood cells were seen in pulmonary capillaries, in alveoli and, in rare observations, extravasated into the interstitium.[14] That many of the erythrocytes had been subjected to osmotic stress was reflected in aberrent red cell profiles; some of these were swollen while others conformed to the contours of adjacent endothelium or exhibited angular configurations. The cardinal ultrastructural pathologic changes seen were intra-alveolar hemorrhage, atelectasis, interstitial edema and endothelial swelling (Figure 3).

Scanning Electron Microscopy

By scanning microscopy at low magnification, the relationships among contiguous alveoli were seen. Pulmonary areas corresponding to those in which cytopathology was observed by transmission or light microscopy showed focal pathology, as evidenced by fully opened alveoli adjacent to atelectatic areas. Alveoli in control tissues were

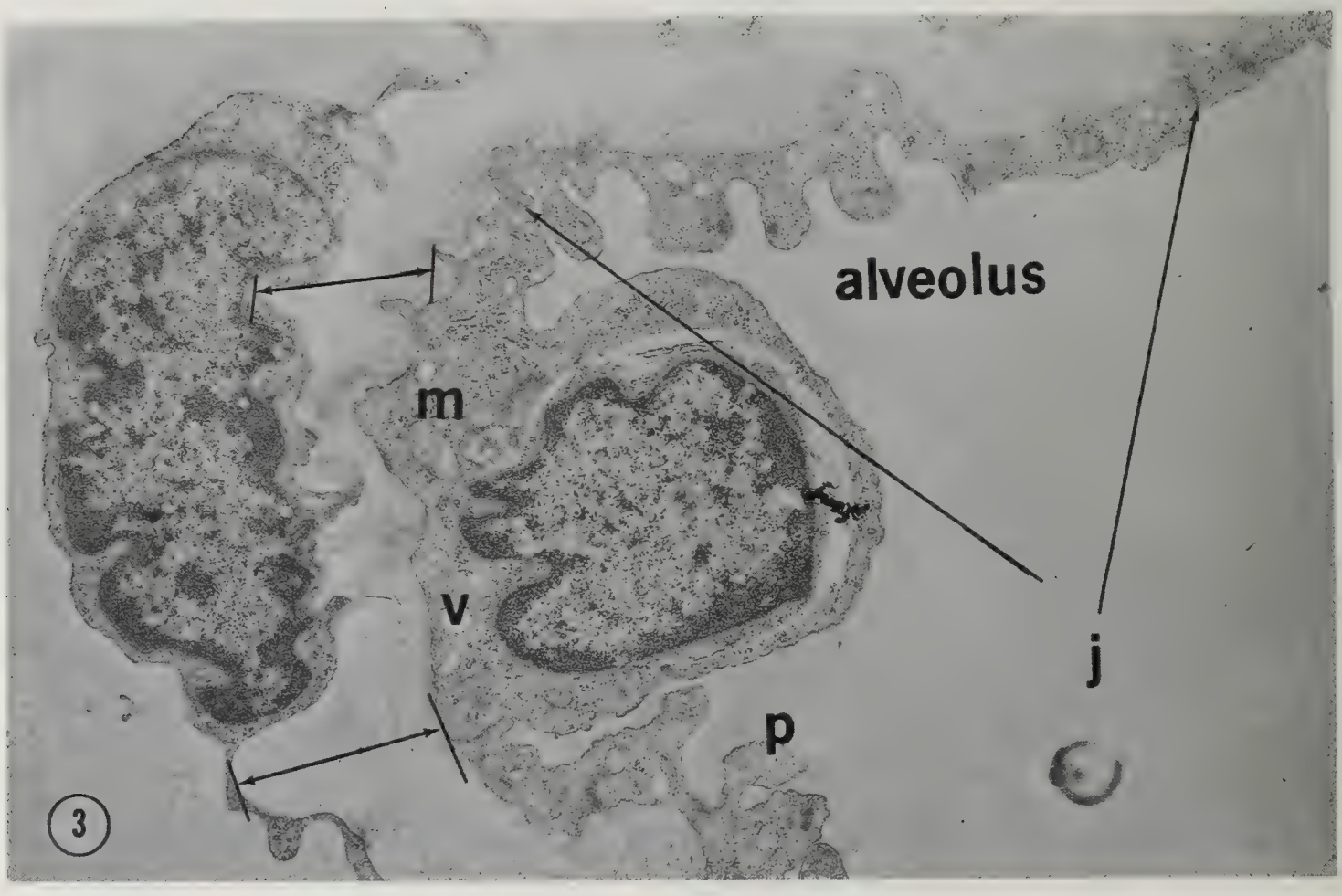


FIGURE 3.—Transmission electron photomicrograph of lung tissue from group III (continually overloaded) dog. Type I epithelial cells are joined at J and show intense pinocytotic activity (p) and vacuolization (v). Marked widening of the interstitial space by edema fluid is seen between brackets. Hypoxemia is reflected by swollen mitochondria (m). ×15,000.

uniformly patient. In micrographs of single alveoli from control dogs, type I and II alveolar epithelial cells, alveolar pores of Kohn and alveolar macrophages were identified. Reduction in the diameter and number of pores of Kohn in smooth walled alveoli from Group III dogs was observed. The outlines of erythrocytes in the underlying alveolar capillaries were seen in micrographs of control tissue; scanning microscopic observations of lung tissue from Group III, continually overloaded dogs differed in that RBC's were observed also free in the alveoli (Figure 4).

Physiologic Parameters

Observed and calculated physiologic values are presented in Table II for Dog 6962 (Group III). The data are representative of all dogs in this group, all of whom died of acute respiratory insufficiency six hours after continual infusion (100 ml/kg/hr) with lactated Ringer's solution. Especially noteworthy are the values for respiratory mechanics, pH, blood gasses, and hemodynamic variables; each changes consistently as an index of pulmonary edema.

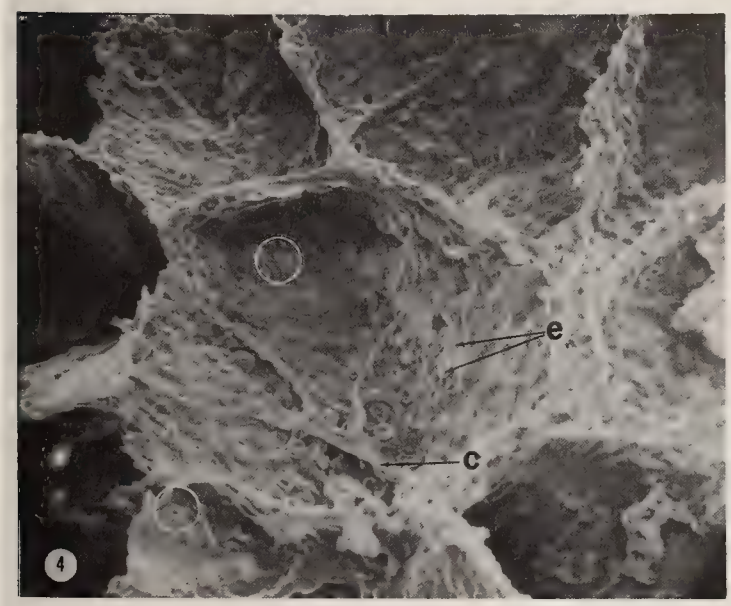


FIGURE 4.—Scanning electron photomicrograph of lung tissue from group III (continually overloaded) dogs.

Erythrocytes (e) are seen free in the alveolus and near a pulmonary capillary (c). Open circles define pores of Kohn. Mag. ×500.

DISCUSSION

Controversy persists in regard to the precise relation between fluid overload and the genesis of acute respiratory insufficiency (ARI).[15] Often, patients who have been given large amounts of electrolyte solutions in resuscitation from low flow states develop an expansion of intravascular and interstitial fluid volumes. Subsequent edema and congestion may result in acute pulmonary insufficiency.

Clinical studies on the genesis of ARI necessarily are compromised by therapeutic management of antecedent trauma and shock; thus, controlled, pathophysiologic assessment of ARI in humans is unlikely since all patients in these situations are treated.[14] An alternative, however, for understanding the clinical conditions may be provided by an animal model of acute respiratory insufficiency. The value of this canine system is that it provides a clinically relevant model of ARI, uncompromised by therapeutic interventions.[16,17] Moreover, the model of fluid overloading is relevant to iatrogenic acute respiratory insufficiency where infusion therapy is used during clinical management.

In these studies, four groups of animals have been investigated. Group I was a control group of 3 dogs in which the effects of the experimental design were evaluated, e.g., anesthesia, immobilization, etc. The dogs were not infused. Group II, consisting of 6 dogs, was infused intermittently (50 ml/kg/hr), 25 minutes each hour for 6 hours. This schedule of infusion did not produce marked ARI; fluid overloading did not exceed diuretic capability, and all dogs survived.

In Group III, both gross and cytopathologic alterations in the lung tissue of continually fluid overloaded dogs and the accompanying changes in respiratory mechanics and hemodynamic variables were consistent, co-correlated indices of acute pulmonary trauma. Only animals in this group showed clinical manifestations of pulmonary edema terminally and died after 5–6 hours of continual infusion with lactated Ringer's solution (100 ml/kg/hr). That continuous, massive fluid overloading produced acute pulmonary insufficiency was reflected in the increased transpulmonary pressure, increased

Series 3 Dog 6962, Male, 26.5 Kgs.

TIME	Hematocrit, %	Urine Volume, ml/hr.	Inspiratory Pres., cm H_2O	Tidal Volume, ml.	Compliance, L/cm H_2O	Respiration Rate, Br/min.	Minute Volume, L/min.	Total Work per Breath, Kg - M/Br	Total Work per Liter, Kg - M/L	Res. Work per Breath, Kg - M/Br	Res. Work per Liter, Kg - M/L	Total Work per min. Kg - M/min.	Res. Work per min. Kg - M/min.	Urine Osmolarity, mOsm	Serum Osmolarity, mOsm	Ph	PO_2, mm Hg	PCO_2, mm Hg	O_2 Saturation, %	Ht. Rate, bpm	Aortic Pressure, mm Hg	Mean Aortic Pressure, mm Hg	Cen. Ven. Pressure, cm H_2O
t_0	42.5	----	7.88	390	.0495	11.4	4.434	.055	.1252	.0072	.0163	.5551	.0723	413	303	7.35	84.1	39.1	92	150	200/105	135	- 1.7
t_{30} S*			6.88	313	.0455	14.9	4.651	.0306	.0988	.0023	.0073	.4595	.0340			7.29	86.7	38.5	92.5	165	165/110	135	5.0
t_{30} P+																							
t_{60}	34	800												234	311	7.35	81.2	42.6	92				
t_{90} S			7.10	243	.0342	24.2	5.871	.0228	.1001	.0098	.0431	.5877	.2530			7.30	77.3	47.3	90.5	210	145/110	125	6.0
t_{90} P																							
t_{120}	30.5	1900												280	310	7.29	70.4	48.2	88.5				
t_{150}S			8.04	184.5	.0231	34.0	6.273	.0210	.114	.0099	.0537	.7151	.3369			7.30	65.7	47.2	87.5	180	135/110	120	7.5
t_{150}P																							
t_{180}	31	1650												283		7.26	51.8	48.9	81				
t_{210}S			12.76	124.4	.00975	75	9.331	.0298	.2265	.0209	.1592	2.113	1.491			7.22	30.0	64.0	59	150	170/125	150	13.0
t_{210}P																							
t_{240}		1650												323		7.22	31.4	138.3	36				
																7.289	30.2	98.8					

TABLE II.—*Physiologic Data Representative of Group III Dogs*

respiratory rate and decreased tidal volume (Table II). Other physiologic alterations observed in continually overloaded dogs ranged from initial changes in lung function, cited above, to decrease oxygen saturation and respiratory acidosis. Respiratory mechanics were also compromised by the development of congestion and edema within the lungs, as observed in the present ultrastructure study. The earliest physiologic changes correlated with the cytopathologic observations of edema (e.g., displacement of collagen bundles by edema fluid, vacuolization and thickening of endothelial cells). Edema about the small airways may have increased airway resistance. Figure 4 shows several adjacent alveoli from Group III dogs; noteworthy are the smooth continuous alveolar walls and the reduction in the diameter of the pores of Kohn.[18] This morphology is consistent with edematous tissues which presumably have occluded the pores of Kohn. Companion light and transmission electron microscopic observations confirm edema at the site of this lung tissue sample.

Congestive changes also may have contributed to decreased pulmonary function, causing both a decrease in compliance and an increase in airway resistance.[19] Similarly, compliance may have fallen because congestion of the alveoli led to changes in surface forces and alveolar collapse. The latter observation was substantiated in scanning electron microscopic observations which demonstrated focal atelectasis, especially in the lower lobes, of lungs from continuously overloaded dogs. Any decrease in lung compliance and increase in airway resistance, whether due to congestion or edema, will increase the energy cost of breathing. Thus, specific indices of pulmonary function, e.g., transpulmonary pressure and the resistive work of breathing, will reflect the observed edema and congestion.

All of the effects caused in Group III dogs were abrogated in the five Group IV animals, which were given furosemide (0.8 mg/kg) every 2 hours, during infusion.

It is significant that in this study with normal dogs, it was necessary to infuse massive volumes of fluid over six hours in order to produce respiratory insufficiency. The dogs developed pulmonary edema only when the retained fluid equalled one-fourth of their body weight. It is most probable, however, that the degree of acute respiratory insufficiency can be induced with a smaller total volume of infusate in dogs, whose pulmonary circulation has been previously compromised. Thus, these observations and the associated canine model of ARI induced by con-

tinuous fluid overloading may provide an experimental system for a better understanding of iatrogenic clinical respiratory failure.

SUMMARY

Pathophysiologic assessment has been made of a canine model of fluid overload-induced acute respiratory insufficiency.

Light microscopy and both transmission and scanning electron microscopy, and salient physiologic analyses, were performed on dogs following fluid overloading.

Only animals continually fluid overloaded (100 ml/kg/hr) showed clinical signs of pulmonary edema and died after 5–6 hours of infusion. Dogs which were controls, intermittently infused (50 ml/kg/hr, 25 minutes each hour for 6 hours) or continually infused (100 ml/kg/hr for 5–6 hours) *and* treated with furosemide, exhibited no clinical signs of acute respiratory insufficiency and survived.

The pathophysiologic observations and the associated canine model of acute respiratory insufficiency induced by continuous fluid overloading may provide an experimental system for a better understanding of iatrogenic clinical respiratory failure.

REFERENCES

1. Berman, H. J. and Fulton, G. P. The microcirculation as related to shock. In *Shock and Hypertension,* 12th Hahnemann Symposium.
2. Blaisdell, F. W., Lim, R. C. and Stallone, R. J. The mechanism of pulmonary damage following shock. Surgery, 130:15, 1970.
3. Daniel, R. A. and Cote, W. R. "Wet Lung," an experimental study. The effects of trauma and hypoxia. Ann. Surg. 127:836, 1948.
4. Pomeranz, M. and Roseman, B. J. Experimental shock lung model. J. Trauma, 8:782, 1968.
5. Wilson, J. W., Ratliff, N. B. and Hackel, D. B. The lung in hemorrhagic shock. Amer. J. Path. 58(2):337, 1970.
6. Coalsen, J. J., Hinshaw, L. B. and Guenter, C. A. The pulmonary ultrastructure in septic shock. Exp. Molecular Path. 12:84, 1970.
7. Ashbaugh, D. G. and Vzawa, T. Respiratory and Hemodynamic Changes After Injection of Free Fatty Acids. J. Surg. Res. 84:417, 1968.
8. Veith, F. J., Hagstrom, J. W., Panossian, A., Nehisen, S. L., and Wilson, J. W. Pulmonary microcirculatory response to shock, transfusion and pump-oxygenator procedures: a unified mechanism underlying pulmonary damage. Surgery, 64:95, 1968.
9. Altschule, M. D. *Acute Pulmonary Edema.* Modern Medical Monographs. Grune and Stratton, New York, 1954.
10. Paine, R., Howard, F. A., Butcher, N. R. and Smith, J. R. Observations on pulmonary lymph flow and edema. Fed. Proc. 8:123, 1949.
11. Smith, J. R. Observations on Experimental Pulmonary Edema. J. Clin. Investigation, 28:802, 1949.
12. Butcher, H. R., Smith, J. R., and Howard, F. A. Observations on the role of pulmonary congestion in the production of edema of the lungs. J. Lab. & Clin. Med. 36:288, 1950.
13. Molokhia, F. A., Robinson, W. J., Martin, J. E., Liss, R. H., Ballantine, T. V. N. and Norman, J. C. Canine models of acute respiratory insufficiency (ARI) with fluid overload. Clin. Res., 19(4): 741, 1971.
14. Teplitz, C. The ultrastructural basis for pulmonary pathophysiology following trauma. J. Trauma, 8: 700, 1968.
15. Thal, A. P. *Shock, A Physiologic Basis for Treatment.* Yearbook Publishers, Chicago, 1971.
16. Daly, B. D. T. and McNary, W. F. Type II (granular alveolar) penumocyte in ultrastructural analysis of stressed human lung. Surgical Forum, 27: 38, (1971).
17. McNary, W. F. and Berger, R. L. Ultrastructure of pulmonary edema in man. J. Clinical Res. 19(4): 509, 1971.
18. Nowell, J. A. and Tyler, W. S. Scanning electron microscopy of the surface morphology of mammalian lungs. Amer. Rev. Resp. Dis. 103: 313, 1971.
19. Freeman, J. and Nunn, J. Ventilation-perfusion relationships after hemorrhage. Clin. Sci., 24:135 1963.

MEDIAL HYPERPLASIA OF THE PULMONARY ARTERIES OF CATS

T. W. Swerczek*

Medial hyperplasia of the pulmonary arteries (MHPA) of cats is a common finding, especially in older animals. The lesion is found naturally in approximately one-third of the cats. A majority of older cats have mild to marked lesions of MHPA.

The exact mechanism which triggers medial hyperplasia and hypertrophy of the smooth muscle cells of the vascular medial layer is unknown. However, the lesion appears to be associated by the presence and migration of nematode parasites in the lungs. MHPA was induced experimentally in 100% of the cats infected with *Aelurostrongylus abstrusus* and *Toxocara cati* which are natural parasites of cats. The lesion was also induced by *T. canis* and *Ascaris suum*.

In cats infected with *T. cati*, lesions of MHPA appeared 3 weeks post-infection and the arteries were very thickened by 6 weeks. The increased thickness of the pulmonary arteries was due primarily to hyperplasia of the medial myocytes. During the development of the lesion, myocytes were hypertrophied and the cytoplasm frequently contained pink droplets when stained with hematoxylin and eosin. A proliferative endarteritis and a periarteritis characterized by the presence of many eosinophil leukocytes were common findings in the acute disease but were less frequently observed in the chronically infected cats.

One of the causes of MHPA appears to be related to the migration of nematode parasites through the lungs. Since the lesions in the acute stages are suggestive of an allergic response, it is postulated that MHPA is initiated by local anaphylaxis induced by a parasitic antigen-antibody reaction. Other non-nematode antigens may act similarily; however, none have been identified.

Since MHPA can be experimentally induced in 100% of the cats infected with *T. cati* and *A. abstrusus*, the cat is an unique model for studying the mechanisms of arterial hyperplasia and pulmonary hemodynamics.

INTRODUCTION

Medial hyperplasia of the pulmonary arteries (MHPA) is commonly observed in cats. The lesion is more frequently seen in older cats but is also present in the young. No apparent breed or sex predilection or obvious clinical disturbances have been associated with the lesion.

MHPA was first reported by Campbell in cats exposed to low oxygen pressure.[1] Others have verified the presence of the lesion in cats.[2-8]

Neumann et al. observed MHPA in cats that were given metrazol and insulin by injection.[9] Kell et al. noted MHPA following excitation of the brain with electrical impulses and with metrazol by injection.[10] Recently, MHPA could not be induced with metrazol[11] or with serotonin.[12] It was concluded that the earlier workers used cats that already had the lesion.

The first association between *Aelurostrongylus abstrusus* and MHPA was made in 1938 by Pritchett.[13] This relationship was subsequently reported by others.[14-19] Hamilton[20] and Swerczek[21] experimentally reproduced MHPA with *A. abstrusus*. Swerczek et al. reported that MHPA could be induced experimentally with *Toxocara cati* as well as other nematodes that migrate through the lungs of cats.[22]

Rohovsky et al. found MHPA in 6 of 95 germfree cats.[23] Rogers et al. found the lesion in conventional and specific-pathogen-free cats.[24] However, the arterial thickness of the pulmonary arteries illustrated in the latter paper was very mild when compared with that of the experimentally induced lesion. Until recently, the cause of MHPA was unknown and it could not be experimentally reproduced. The purpose of this paper is to describe how MHPA can be induced experimentally and to describe the possible pathogenesis of MHPA.

In the author's experience, all cats that were naturally infected with *A. abstrusus* had MHPA. However, the lesion was frequently observed in cats that had no evidence of previous exposure to *A. abstrusus*. It was postulated that other nematodes that migrate through the pul-

* Department of Verterinary Science University of Kentucky Lexington, Kentucky.

monary tissues as part of their life cycle may induce MHPA as *A. abstrusus*.

MATERIALS AND METHODS

Young cats were used to help insure against having the natural lesion and were either obtained as kittens or were raised in the laboratory. They were caged on wire to prevent ingestion of parasites and were 10 to 12 weeks of age at the start of the experiment. They were divided into 6 groups and were infected and sequentially killed to study the development of MHPA.

Group I. Fifty-nine cats were infected with 1,000 infective *A. abstrusus* larvae and were infected for 1 to 137 weeks.

Group II. Thirty-three cats were infected with 2,000 *T. cati* infective eggs/week. They were killed after 2 to 74 weeks of infection.

Group III. Fourteen cats were infected with 4,000 infective *T. canis* eggs/week. They were killed after 3 to 18 weeks of infection.

Group IV. Twelve cats were infected with 4,000 *Ascaris suum* infective eggs/week and were killed after 3 to 32 weeks of infection.

Group V. Nine cats were infected with 4,000 *Toxascaris leonina* infective eggs/week and were killed after 9 weeks of infection.

Group VI. Thirty-one cats were raised the same as the experimental groups but were not exposed to parasites.

Lungs were fixed in 10% buffered formalin. Tissues were embedded in paraffin, cut at 6 microns and stained with hematoxylin and eosin. The following special stains were performed on lung sections: Verhoeff's elastic stain, Gomori's trichrome, oil-red-O, and periodic acid-schiff-reaction.

RESULTS

Group I, Aelurostrongylus abstrusus. The first arterial lesions appeared at 6 weeks post-infection (PI). Endothelial swelling, subendothelial vacuolation and mild myocyte hyperplasia were the first changes observed. The tunica media increased in thickness by 8 weeks PI and was very thick by 12 to 14 weeks and appeared to reach a maximum thickness at 40 weeks PI (Figures 1 and 2). Occasionally, mitotic cells were present and many myocytes contained vacuoles with droplets that stained pink with H & E and were negative with the PAS reaction and oil-red-O stain. Medial myocytes were commonly orientated irregularly and some occurred in fascicles. The affected arteries were very tortuous. The lumens of the vessels appeared almost occluded in some arteries.

Endarteritis was mild in most cases and was characterized by endothelial proliferation, subendothelial vacuolation along with a scattering of eosinophil leukocytes. The internal elastic membrane was fragmented in some vessels.

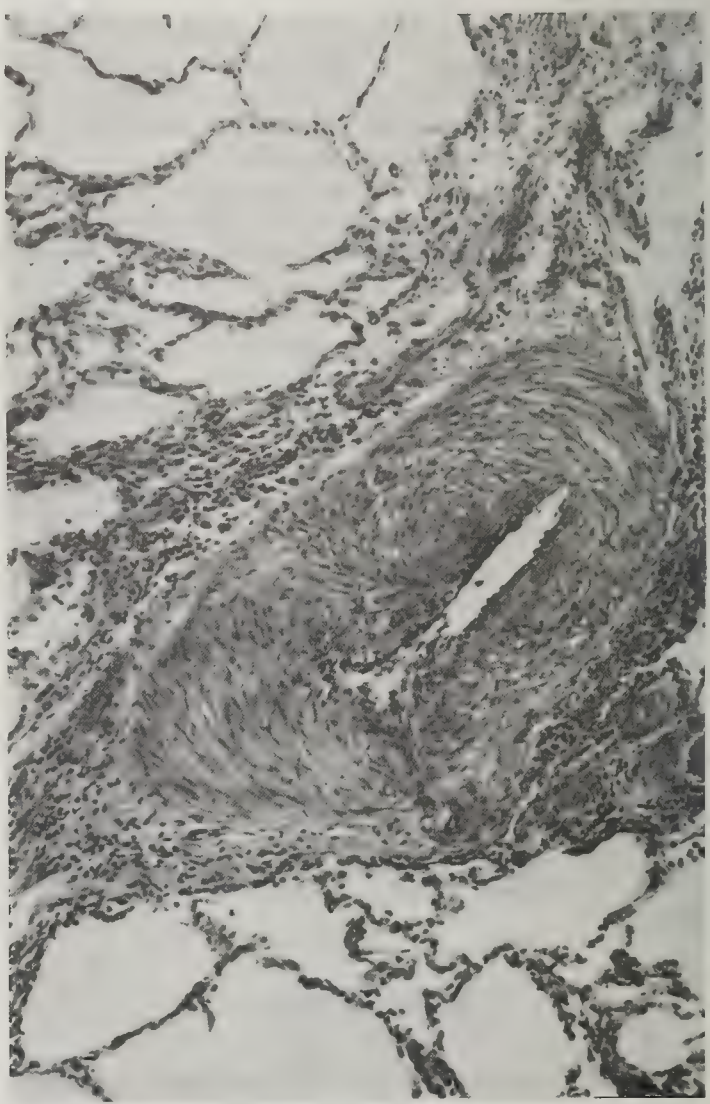


FIGURE 1.—Lung section from a cat infected with *Aelurostrongylus abstrusus* for 118 weeks. A pulmonary artery with a thickened media and mild periarteritis. H. and E stain; × 160.

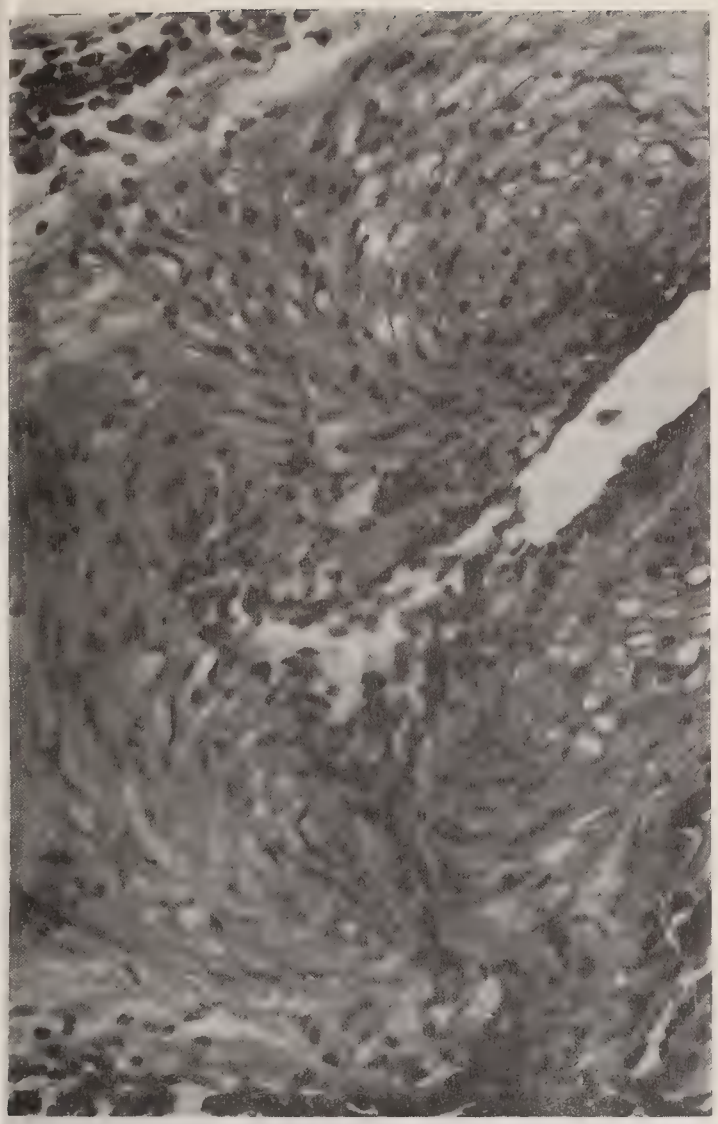


FIGURE 2.—Higher magnification of a pulmonary artery showing hyperplasia of the media, vacuolation of the myocytes, chronic endarteritis and periarteritis. H and E stain; × 400.

MHPA was found in all cats infected with *A. abstrusus* for 6 weeks or more.

Group II, T. cati. Medial hyperplasia of the pulmonary arteries was a consistent finding in cats infected 3 weeks or more. At 3 weeks PI mild endothelial swelling and proliferation, subendothelial vacuolation, medial myocyte hyperplasia, perivascular edema and periarteritis were observed. By 5 weeks PI, the tunica intima and media were thickened. The endothelial cells were hyperplastic and very irregular and the subendothelium contained fibroblasts, many eosinophil leukocytes and vacuoles. The internal elastic membrane was occasionally fragmented. Periarteritis was common and characterized by the presence of edema, plasma cells, lymphocytes and many eosinophil leukocytes. The tunica media was very hyperplastic by 10 weeks PI and gradually increased to a maximum thickness at approximately 34 weeks PI, reaching a pronounced thickness of 45 to 50 cell layers (Figures 3 and 4).

Endarteritis and periarteritis were less frequently observed in cats exposed for more than 16 weeks. The lumens of the affected vessels appeared obliterated in some cases and the arteries were very tortuous.

MHPA was found in all cats infected for 3 weeks or more with *T. cati.*

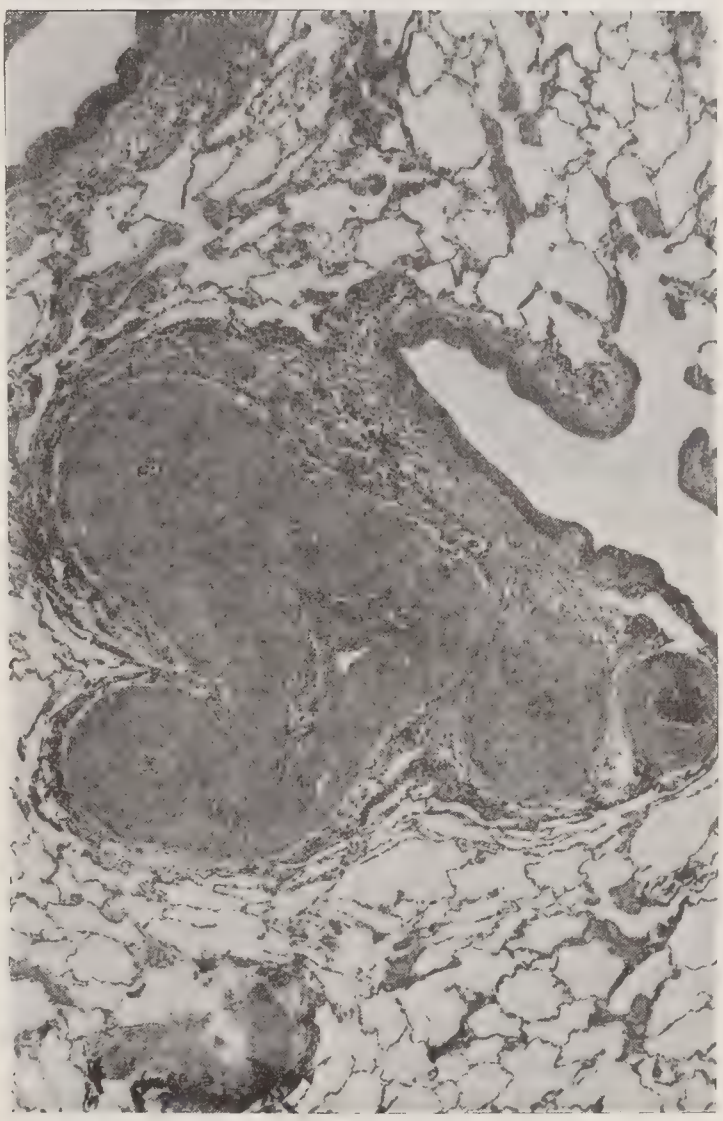


FIGURE 3.—Pulmonary artery from a cat infected with *Toxocara cati* for 52 weeks. Endarteritis and marked hyperplasia of the tunica media. H and E stain; × 64.

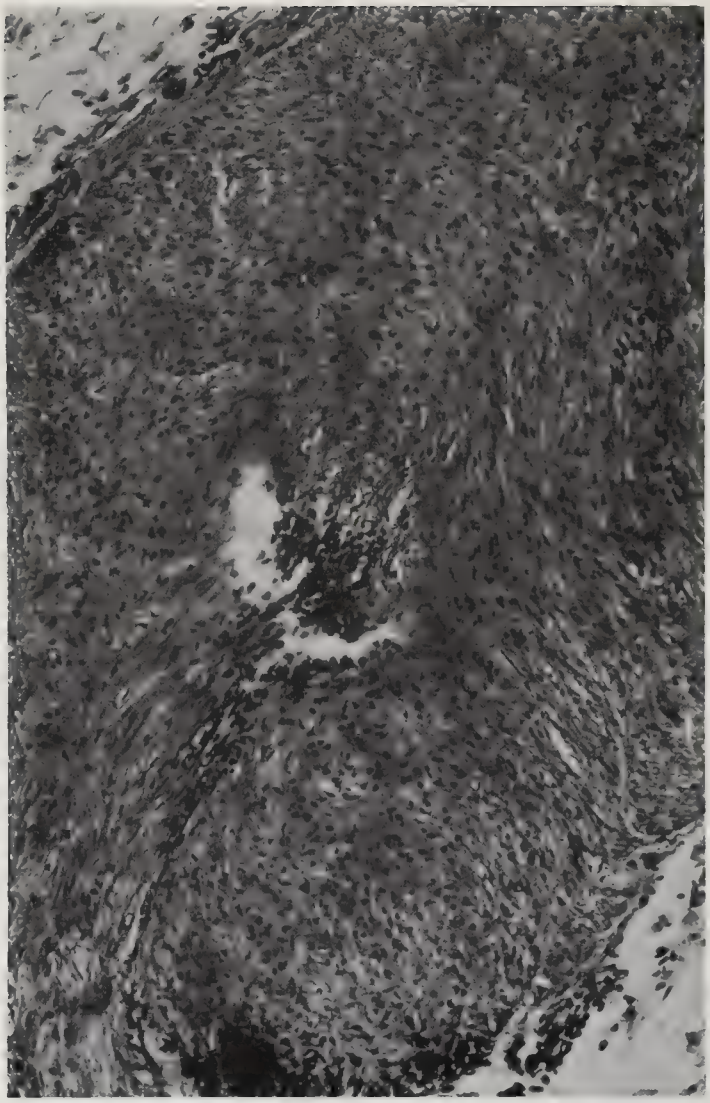


FIGURE 4.—Pulmonary artery from a cat infected with *T. cati* for 74 weeks. An endarteritis and a marked thickening of the tunica media. H and E stain; × 160.

Group III, T. canis. The lesions in the pulmonary arteries were similar to those induced by *T. cati* except endarteritis and periarteritis were more severe. The pulmonary arteries were very thickened by 6 to 8 weeks PI (Figure 5). MHPA was induced in 11 of the 14 cats exposed to *T. canis.* In the 11 cats with MHPA there were parasitic granulomas in the lungs and other organs which indicated the cats had become infected. In 3 cats there was no evidence of parasitic granulomas in the lungs or other tissues, which suggests that the parasite did not migrate. In these cats, the pulmonary arteries were normal. *T. canis,* unlike *T. cati,* induced numerous parasitic granulomas in the lungs and other tissues.

Group IV, Ascaris suum. MHPA was induced in 10 of 12 cats infected with *A. suum.* The lesions were much milder than *T. cati* and *T. canis.* In two cats there was no evidence that the larvae had migrated, and the pulmonary arteries were normal.

Group V, T. leonina. All cats infected with *T. leonina* had normal pulmonary arteries. There was no evidence of parasitic migration in any of the lungs, and MHPA was not observed. This parasite is restricted to the gastrointestinal tract and does not migrate through the lungs.

Group VI, Controls. Four of the 31 control

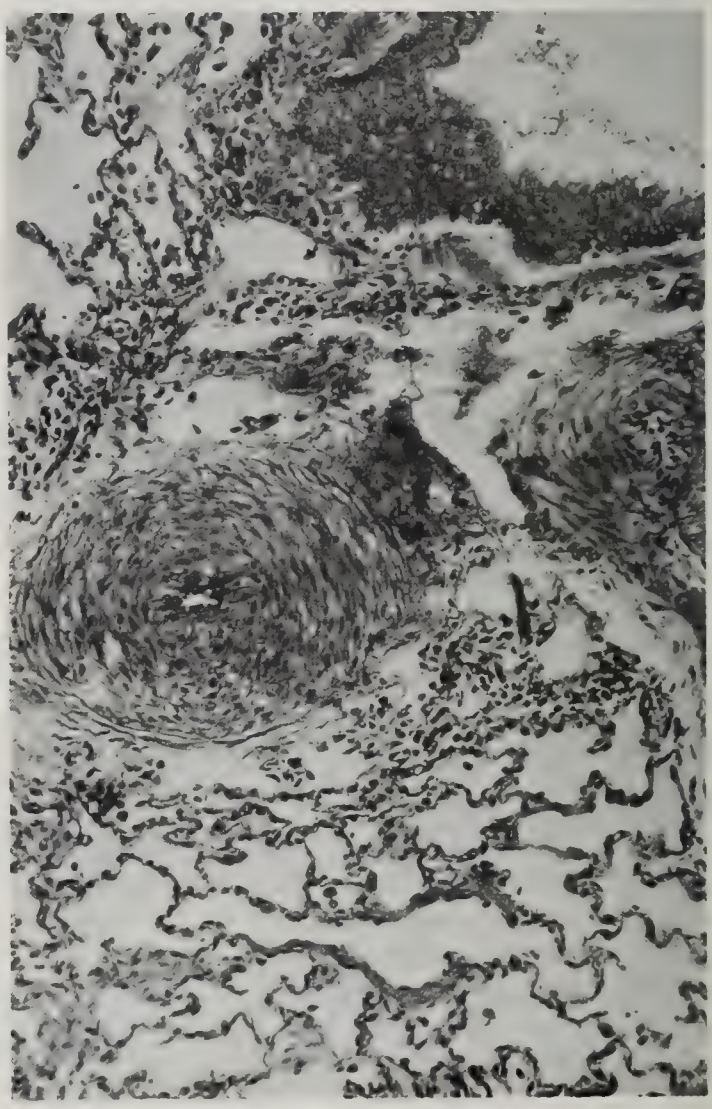


FIGURE 5.—Pulmonary arteries from a cat infected with *Toxocara canis* for 8 weeks. Thickened pulmonary arteries and periarteritis. H and E stain; × 160.

cats had mild MHPA, but in all cases it was very mild and only a few vessels were affected. The thickest vessels had only up to 8 cell layers in the tunica media as compared with a common finding of up to 45 to 50 cell layers in the experimental groups.

DISCUSSION

Medial hyperplasia of the pulmonary arteries can be induced experimentally in up to 100% of the cats that have parasites migrating through the lungs. The most common cause of the lesion in naturally infected cats is probably *T. cati* and *A. abstrusus*. MHPA was also observed in cats that probably were not exposed to pulmonary parasites, but in these cats the thickness of the pulmonary arteries was very mild when compared with the lesion seen in the experimental cats as well as some cats with the natural lesion. The MHPA induced experimentally with *A. abstrusus* and *T. cati* was identical to the lesion seen in naturally affected cats. The mechanism which triggers the medial myocytes to proliferate is unknown. Since MHPA becomes evident approximately 3 weeks, PI, a local anaphylaxis resulting from an antigen-antibody reaction may be involved. The many eosinophil leukocytes seen in the tunica intima, adventitia and occasionally in the media and the eosinophil leukocytes, plasma cells and lymphocytes seen in the tunica adventitia during the acute stages of the lesion suggest an antigen-antibody response.

Mediators associated with the antigen-antibody reaction, such as histamine, are powerful vasocontrictors and may initiate arterial spasms. These spasms may cause the medial myocytes to proliferate. Since more than one pharmacologic mediator acting in a complex fashion is associated with anaphylaxis, it would be unlikely that MHPA could be experimentally produced by a single mediator such as histamine.

The mild lesions seen in the control cats may be a normal anatomical difference in cats, or possibly other allergins in the environment may act as less potent antigenic stimuli to the lung. McCusker and Aitken suggested that the lung is the shock organ of the cat.[25] In light of the foregoing studies, possibly the pulmonary arteries should be considered the shock organ of the cat. Cats with MHPA have a mild pulmonary hypertension.[26] Since MHPA can be consistently induced experimentally in cats infected with *T. cati* and *A. abstrusus*, the cat should be a useful model for the study of pulmonary hemodynamics.

SUMMARY

Medial hyperplasia of the pulmonary arteries was induced experimentally in cats by *Aelurostrongylus abstrusus, Toxocara cati, T. canis* and *Ascaris suum*. It appears that MHPA can be induced by any nematode that migrates through the lungs of cats, but in natural cases *T. cati* and *A. abstrusus* are probably the most important cause. The increased thickness of the pulmonary arteries seen in a few of the control cats was very mild as compared with that achieved in the experimental cats and with the marked lesions seen in some natural cases.

The mechanism that triggers hyperplasia of the medial myocytes is unknown. Since the lesion in the acute stages is suggestive of an allergic response, it is theorized that the lesion is the result of an antigen-antibody response. Mediators released during the antigen-antibody reaction may initiate arterial spasms and subsequent myocyte hyperplasia.

Since MHPA can be consistently induced experimentally in cats with *T. cati*, as well as *A. abstrusus*, the cat may be a useful model to study pulmonary hemodynamics.

ACKNOWLEDGMENTS

The investigation reported in this paper (No. 72-4-8) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director. Experimental work described in this paper was completed while the author was at the University of Connecticut, Storrs, Conn.

REFERENCES

1. CAMPBELL, J. A. Further observations on oxygen acclimatization. J. Physiol. 63:325–342, 1927.

2. Rubarth, S. Lokale hyperplastische prozesse in der art. pulmonalis der katze. Skand. Vet. Tidskr. 30:489–514, 1940.
3. Marcato, A. Arteriosclerosi intimale nel polmone del gatto. Nuova Vet. 18:75–83, 1940.
4. Olcott, C. T., Saxton, J. A., and Modell, W. Medial hyperplasia in pulmonary arteries of cats. Amer. J. Path. 22:847–853, 1946.
5. Martin, J. Über das vorkommen von sklerosierenden prozessen in den asten der arteria pulmonalis bei katzen. Mh. Vet. Med. 14:692–695, 1959.
6. Dahme, E. Der gëstaltwandel der lunge und arteriellen lungenstrombahn bei störungen des kleinen kreislaufes. Berl. Münch. tierärztl. Wschr. 73:333–336, 1960.
7. Scratcherd, T., and Wright, D. E. Medial hypertrophy and hyperplasia in cat pulmonary artery. Arch. Path. 72:703–708, 1961.
8. Tashjian, R. J., Das, K. M., Palick, W. E., Hamlin, R. L., and Yarns, D. A. Studies on cardiovascular disease in the cat. Annals of the New York Academy of Sciences. 127:581–604, 1965.
9. Neumann, M. A., Cohn, R., and Katzenelbogen, S. Experimental "shock therapies" in cats with insulin and metrazol. Amer. J. Psychiat. 98:668–672, 1942.
10. Kell, J. F., Jr., Hennigar, G. R., and Hoff, E. C. Experimental medial hypertrophy and hyperplasia of cats' pulmonary arteries. Arch. Path. 61:239–244, 1956.
11. Hamilton, J. M. The effect of metrazol on the pulmonary arteries of the cat. J. Path. Bact. 91:634–636, 1966.
12. Hamilton, J. M. The effect of serotonin on the pulmonary arteries of the cat. J. Path. Bact. 91:249–252, 1966.
13. Pritchett, H. D. Lung worms in a kitten. J. Amer. vet. med. Ass. 92:692–694, 1938.
14. Blaisdell, K. F. A study of the cat lungworm, *Aelurostrongylus abstrusus*. Thesis, Cornell University, 1952.
15. Mackenzie, A. Pathological changes in lungworm infestation in two cats with special reference to changes in pulmonary arterial branches. Res. vet. Sci. 1:255–259, 1960.
16. Hamilton, J. M. *Aelurostrongylus abstrusus* infestation of the cat. Vet. Rec. 75:417–422, 1963.
17. Hamilton, J. M. Pulmonary arterial disease of the cat. J. Comp. Path. 76:133–145, 1966.
18. Schiefer, B., and Hänichen, T. Über die durch *Aelurostrongylus abstrusus* Railliet 1898 verursachte pneumonie bie katzen. Tierärztliche Wochenschrift 13:243–246, 1966.
19. Stünzi, H., Teuscher, E., and Pericin-Rauhut, D. Die hyperplasie der arteria pulmonalis bei feliden. Path. Vet 3:461–473, 1966.
20. Hamilton, J. M. Experimental lungworm disease of the cat. Association of the condition with lesions of the pulmonary arteries. J. Comp. Path. 76:147–157, 1966.
21. Swerczek, T. W., Ph. D. Thesis, The University of Connecticut, 1969.
22. Swerczek, T. W., Nielsen, S. W., and Helmboldt, C. F. Ascariasis causing pulmonary arterial hyperplasia in cats. Res. Vet. Sci., 11:103–104, 1970.
23. Rohovsky, M. W., Griesemer, R. A., and Wolfe, L. G. The germfree cat. Lab. Anim. Care 16:52–59, 1966.
24. Rogers, W. A., Bishop, S. P., and Rohovsky, M. W. Pulmonary artery medial hypertrophy and hyperplasia in conventional and specific-pathogen-free cats. Amer. J. Vet. Res. 32:767–774, 1971.
25. McCusker, H. B., and Aitken, I. D. Anaphylaxis in the cat. J. Path. Bact. 91:282–285, 1966.
26. Jonas, A. M., Swerczek, T. W., and Downing, S. E. A pathogenetic study of medial hypertrophy and hyperplasia of pulmonary vessels in the cat. Am. J. of Path. Submitted for publication, 1972.

DISCUSSION

G. E. Bisgard, University of Wisconsin, Madison: Has anyone measured pulmonary artery pressure in these cats? Also, has anyone tried to simply inject antigen into the lung, trying to develop the lesion that way?

Dr. Swerczek: I have injected several antigens without success in cats. I personally haven't measured the pulmonary arterial pressure in these cats, but Dr. Jonas at Yale has done considerable work with this.

ALVEOLAR, BRONCHIOLAR AND PULMONARY VASCULAR RESPONSES OF ISOLATED RAT AND GUINEA PIG LUNGS

Frederick Sperling,* William L. Marcus** and A-A. O. Coker*

Continuously perfused, isolated rat and guinea pig lungs, which breathe by negative pressure, and which may be injected with single doses of drugs, permit distinction between alveolar and bronchiolar effects. This distinction is based on the pulmonary vascular differences, between the species, in anastomoses between pulmonary and bronchial arteries. The differences in response between the pulmonary and the systemic vasculature may also be distinguished. Barbiturates have been shown to be concentrated by the lungs of both species, to be retained for long periods of time, and to exert profound peripheral inhibitory effects on the capability of the lungs to inflate. Differences in the two species in response to the barbiturates are small. The two species differ in their response to autonomic drugs. Pulmonary artery injection in guinea pig lungs appear to reach the bronchioles and to affect them. In the rat, the primary effect appears to be on the alveoli, and it is doubtful that the bronchioles are reached. The pulmonary vasculature of the rat lung does not respond in the same manner as does the systemic vasculature. Acetylcholine constricts; norepinephrine induces progressively lesser constriction with repetitive or increasing doses; isoproterenol dilates. The actions of each of these can be blocked by the appropriate blocking agent. All of the barbiturate and autonomic effects noted may be reversible or irreversible, and appear to be dose-related.

INTRODUCTION***

It is now possible in isolated perfused pulmonary systems to distinguish alveolar responses from those of the tracheobronchiolar tree. The tidal volume increases or decreases, which may be seen in response to pharmacologically active agents, may be due to changes in airway patency, alveolar expansibility or both.

It is now also possible in such systems to simultaneously and independently observe changes in the pulmonary vascular system. Variations in perfusion flow rate reflect vascular constriction or dilatation. Pulmonary vascular responses may differ from those of the systemic vasculature.

It can now be shown *in vitro* and *in vivo* that the pulmonary system has functions other than those associated with the exchange of gases and other substances between the environment and the circulation. Lungs can concentrate many circulating substances and retain them for long periods of time. Such retained substances, and probably others in the circulation as well, may have significant peripheral effects on the pulmonary system.

The pulmonary system may be divided into three morphologically and physiologically distinct parts. The airways are for the most part lined internally with a pharmacologically reactive and secreting epithelium. They are covered, for the most part, with a pharmacologically reactive smooth muscle coat. They open into the alveoli which are walled by thin partitions consisting of capillary endothelium, two types of scattered epithelial cells, one of which secretes the pulmonary surfactant, scattered Mast cells in many species, scattered histiocytes, and scattered red and white blood cells. There are no smooth muscle fibers. Aside from the Mast cells and the surfactant-secreting epithelial cells, little pharmacological attention has been paid to the other elements and to the alveolus as a unit.[1]

The alveoli are separated from each other by interalveolar septae through which pass a complex system of capillaries (Figures 1 & 2). These capillaries arise from the pulmonary artery originating from the right ventricle and drain into the pulmonary veins entering the left atrium. These veins also join, via capillaries and anastomoses, with bronchial veins and arteries the arteries arising from the aorta. In some species, such as the rat, large numbers of atrial fibers are found in the walls of the large col-

* Department of Pharmacology, Howard University College of Medicine, Washington, D. C.

** Oakdale Toxicology Center, University of Iowa, Oakdale, Iowa 52319.

*** Supported in part, by USPHS Grant No. EC-00234.

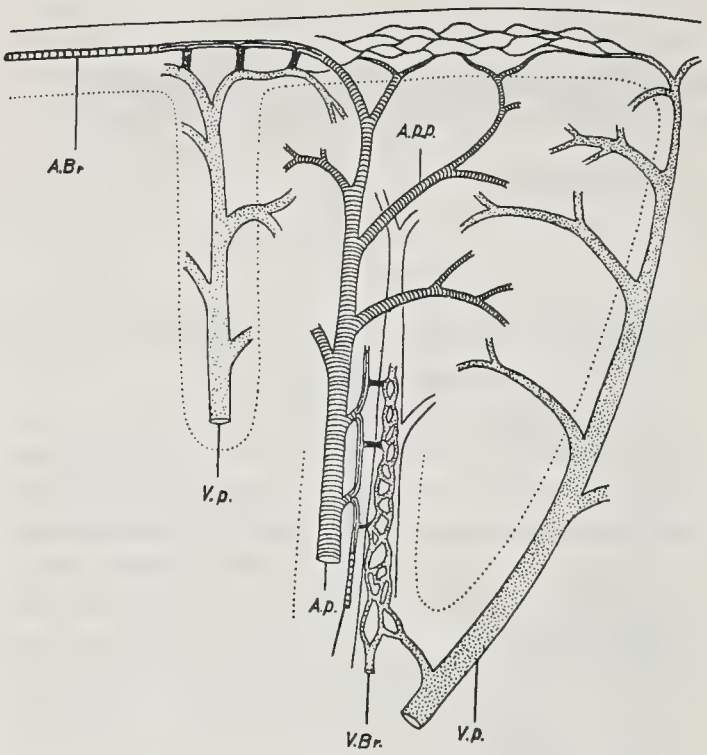


FIGURE 1.—Diagram showing relations between pulmonary and bronchial circulation (after Hayek). See Camarri and Marini.[3]
A.p. = Pulmonary artery
V.p. = Pulmonary vein
A.Br. = Bronchial artery
V.Br. = Bronchial vein
A.pp. = Pleural aborization of the pulmonary artery

lecting veins. In other species, such as the guinea pig, such fibers are found only at the base of the veins as they enter the atrium.[2]

The airways are vascularized by the bronchial arteries which arise at or just proximal to the aortic arch.[3] The bronchial artery probably does not differ pharmacologically from other systemic arteries. In many species this artery anastomoses with the pulmonary artery. In guinea pigs (and in man and horse), the anastomoses are profuse and widely disseminated[4] (Figures 1 and 2). In other species, such as the rat, they are scarce and limited to the hilar region.[3,4] In still other species, such as the cat and monkey, they are absent.[4] These differences in circulation make it possible to differentiate between airway and alveolar responses by injection into either the pulmonary artery or the bronchial artery or by the nature of the response to pulmonary artery injection. In cat and monkey lungs, it may be possible to distinguish not only between alveolar and bronchiolar responses, but simultaneously between pulmonary and systemic vascular responses.

The autonomic responses in the three divisions also seem to be separate. The pulmonary arteries have shown differing reactions to acetylcholine resulting from both species differences and differences in experimental techniques.[5] Some experiments have shown vaso-

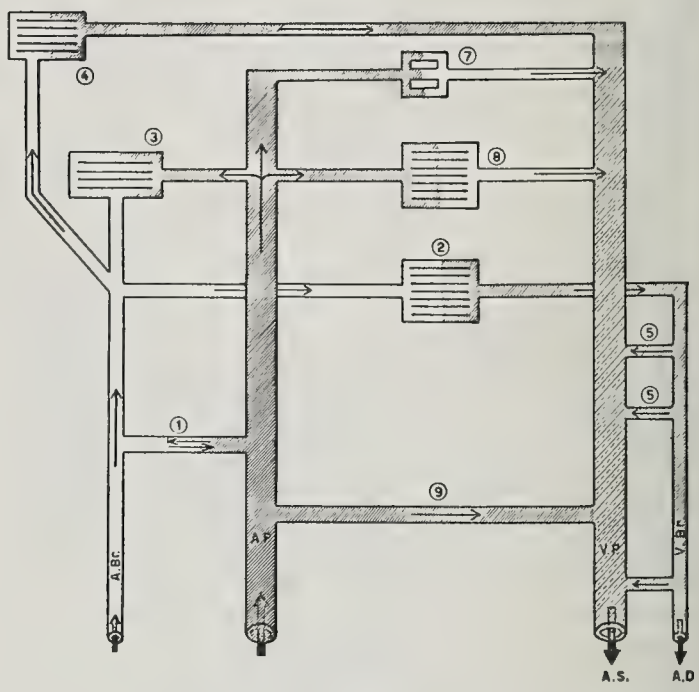


FIGURE 2.—Diagrammatic reconstruction of the pulmonary circulation in the lungs of a normal adult. See Camarri and Marini.[3]
1: Precapillary arterio-arteriole broncho-pulmonary anastomoses
2: Bronchial capillary path
3: Broncho-pulmonary capillary junction
4: Pleural vascularization circuit of bronchial artery
5: Broncho-pulmonary venous system of Lefort
6: Hilar junction of broncho-pulmonary veins
7: Sub-pleural circuit of giganto-capillaries
8: Pulmonary capillary path
9: Pulmonary arterio-venous anastomoses
A.S. = left atrium
A.D. = right atrium
Other abbreviations as in Fig. 1.
= Arterial blood
= Venous blood
= Mixed blood

dilatation and some vasoconstriction in response to acetylcholine. The bronchial arteries, however, responded as did other systemic arteries. Norepinephrine and isoproterenol were both reported to act on the pulmonary vasculature in the same manner as they did on the systemic vasculature.[5] However, in the case of norepinephrine, there may be some question (See infra).

The *in vivo* action of these drugs on the bronchioles are well known. Acetylcholine produced a slowly developing large reduction in tidal volume in guinea pig lungs.[6] As discussed below, the slow development probably indicated airway response. Similar effects were produced by DFP and paraoxon.[7] Atropine could block but not reverse the effects. Epinephrine reversed the effects. The effects after parathion were questionable. It would have been surprising had parathion had the same action as paraoxon.

In previously published reports on responses of isolated guinea pig lungs to pulmonary artery injections of catecholamines, Bhattacharya[8] found that isoproterenol and epinephrine increased tidal volume, probably by action on the bronchioles. Heymans and Dautrebande[1] confirmed this for isoproterenol and showed, in addition, that isoproterenol placed directly on the pleural surface also induced pulmonary dilatation, thereby demonstrating the independent capability of the alveoli to respond. Neither of these reports included investigations on the responses of the pulmonary vasculature, and all involved the guinea pig only. It thus became necessary to do similar studies with isolated rat lungs, but now the pulmonary vasculature would be studied as well.

The continuously perfused, isolated lung, caused to inflate and deflate by cyclic negative pressure, is uniquely capable of distinguishing the activities of each of these parts of the pulmonary system. In such a system, the lungs inflate and deflate (breathe) only to the extent that they are capable of doing so (Figure 8). Positive pressure systems overlook this capability. Additionally, the system as developed by Delaunois[9] permits drugs to be administered either by perfusion, along with the Tyrode's, or by single, pulmonary artery injection, or via the airway in the form of gases or aerosols. The responses of alveoli and airway to drug administration by pulmonary artery injection or by airway may be seen by changes in tidal volume or by loss of capability to expand (Figure 8). Vascular effects may be seen by changes in perfusion flow rate indicating pulmonary vascular constriction or dilatation. The two types of changes may be reversible or irreversible. When the changes are irreversible, the lungs are no longer considered viable.

MATERIALS AND METHODS

The system[9] consists of a 10 cm^3 transparent chamber, open at top and bottom of one side to an accessory chamber, and at the bottom of another side to a negative pressure pump. The accessory chamber contains an airway segment, a heater and thermostat, and a blower. An attached aneroid manometer monitors chamber pressure. The chamber is sealed with a cover plate through which a continuation of the airway passes. A tracheal cannula hangs at right angles from the airway. Thermistors[10] are mounted in the airway on either side of the tracheal cannula. Temperature differences between the two which develop during lung inflation and deflation are detected by these thermistors and recorded on a polygraph. These differences are directly related to tidal volumes developed at a given negative pressure. Negative pressures cycle 25–27/min from zero to a preset value of 6–8 mm Hg for rat lungs and 8–10 mm Hg for guinea pig lungs. The cover also has a port with a needle valve continuous with a pulmonary artery cannula. Another port leads to a sidearm of the cannula through which injections may be made.

Scrubbed, water-saturated air flowed at 100–200 ml/min at approximately ambient pressure, was warmed in the accessory chamber before flowing through the cover plate. Since the airway diameter is about 10 times that of the tracheal cannula, the airstream was not forced into the trachea. A fair sample, however, entered the lung when it inflated. The airstream could carry vapors and gases and particulates such as aerosols.

Warmed Tyrode's solution, at about 15 cm of water pressure, passed through a rotameter

from a reservoir, through the controlling needle valve and into the pulmonary artery via the cannula. Perfusion was continuous except at the time of injection. Perfusion was stopped at that time to insure that the injection entered the artery directly, rather than into the perfusion reservoir. When necessary, perfusion effluent could be collected through a chamber drain.

Rats and guinea pigs were anesthetized by electroshock. The trachea was exposed and ligated during an inspiration to prevent atalectasis. The animals were then exsanguinated by cutting the carotids. The lungs and heart were then excised and placed in a bath of warm Tyrode's. The pulmonary artery was then cannulated through the right ventricle, and the left ventricle cut away to permit free flow of the effluent, and the trachea slipped over the tracheal cannula and tied. The whole preparation was then sealed into the machine. Tidal volume, at the given negative pressures, was allowed to stabilize before drug administration was begun. Perfusate flow was stabilized at 0.2–0.3 ml/min for rat lungs and 0.3–0.5 ml/min for guinea pig lungs. Higher rates induce edema rapidly. At these rates, edema developed slowly, if at all, and mostly as a result of drug administration. Reduced flow rates were assumed to indicate vascular constriction; increased rate, dilatation. Such vascular responses were absent in non-viable lungs.

Electroshock anesthesia was used because it had been shown by Heymans and his co-workers that after all commonly used anesthetics, inhalation and fixed, lung preparations were either short lived, unresponsive or both (pers. comm.). Consequently, it became of interest to investigate the peripheral pulmonary and pulmonary vascular effects of various barbiturates.

RESULTS AND DISCUSSION

Lungs from guinea pigs weighing 600–800 grams were injected several successive times at 15 minute intervals into the pulmonary artery with equivalent pKa solutions of phenobarbital, pentobarbital, thiopental or hexobarbital (Table I).[10] Both pulmonary and vascular effects were dose-related. Irreversible respiratory cessation was seen at each dose level of each barbiturate, but more successive doses were needed at the lower dose levels. Visible edema, when it developed, occurred late. Transient large tidal volume increases were seen after the second or third of the successive doses of each barbiturate. Eventual, irreversible, large reduction in tidal volume was seen in those lungs which remained viable. There was some evidence that the amount of tidal volume reduction might also be dose-related. In every instance, effects were delayed and developed slowly after the injection.

Rat lungs from animals weighing 250–400 grams were about half the size of guinea pig lungs. Since doses as much as four times greater were required (Table I), such lungs actually received doses as much as eight times greater. Dose-related, persistent tidal volume decreases appeared rapidly; increases were unusual. Respiratory cessation never occurred, but tidal volume was reduced to 80–90% of base volume. More important perhaps, was the observation that successive doses gave progressively lesser responses. In guinea pig lungs, each successive dose elicited a full response.

The delayed but more intense response and the frequent transient tidal volume increases seen in guinea pig lungs suggest that these noticeable effects were predominantly bronchiolar through the profuse anastomoses, in this species, between pulmonary and bronchial arteries. Heymans[1] stated that more than 99%

TABLE I.—*Barbiturate Doses Injected Into the Pulmonary Artery of Isolated Lungs (mg/lung)*

Barbiturate	Guinea Pig	Rat
Phenobarbital	0.30	1.2
	0.15	0.60
	0.075	0.30
		0.15
Pentobarbital	0.10	0.40
	0.05	0.20
	0.025	0.10
Thiopental	0.10	0.40
	0.05	0.20
	0.025	0.10
Hexobarbital	0.60	0.80
	0.30	0.40
	0.15	

of the perfusate in such lung preparations flowed through the pulmonary artery. However, the relatively high injection pressure could have forced retrograde flow through the anastomoses into the bronchiolar circulation.

Pulmonary and bronchiolar arterial blood mix in the capillary bed in humans (Figure 1) and may do so in guinea pigs also.[4] The appearance of the effluent would be as though it were almost completely pulmonary artery circulation. However, bronchiolar effects after pulmonary artery injection would appear after some delay, as they did in guinea pig lungs. The barbiturates could open the airways transiently. Plugging might follow secondarily. Commonly used bronchiodilators have been shown to be frequently associated with secondary respiratory distress and death in man, due to the mucus plugging of the smaller bronchioles.[11] In rat lungs, bronchiolar involvement would be minimal, and the noticeable effects would be predominantly alveolar. These effects would occur quickly, as they did, and would involve the expansion capability or perhaps the elasticity of the alveoli. There would then be a great, but not complete, reduction in tidal volume. These considerations are consistent with the described morphology.

Simultaneously, the pulmonary vascular system generally constricted in guinea pig lungs. Dilatation was infrequent and small. The amount of constriction, as measured by perfusion flow rate, was dose-related. Successive doses gave progressively lesser effects, similar to that seen in the tidal volume responses in rat lungs. The pulmonary vasculature of the rat also showed constriction, but transient dilatation was frequent and only thiopental showed lesser constriction after smaller doses. These *in vitro* responses appear to differ from the generally accepted systemic vascular responses.

These observations indicated that the barbiturates exerted strong and persistent peripheral effects on the bronchioles, the alveoli and the pulmonary vasculature. This in turn suggested that treatment of barbiturate overdosage should include vigorous support of peripheral pulmonary function to augment central stimulation and dialysis. Therefore, it was necessary to know whether persistance of pulmonary peripheral effects were correlated with the presence of barbiturate in the lung.

Pulmonary artery injections of single doses of the four barbiturates (Table II) were administered to rat and guinea pig lungs and the concentration in lung and perfusate determined at various time intervals up to 120 minutes. The lungs remained viable during the entire experiment. These were compared to the concentrations found after various time intervals up to 360 minutes after single intraperitoneal anesthetic doses (Table III). Blood concentrations were also determined. The weights of the guinea pigs and rats were in the same range as noted above.

Barbiturate recovery was confirmed by adding known quantities to homogenized, untreated lung, excised from electroshocked animals. In contradiction to the concentrations later found in experimental lungs, above 90% remained in the lungs despite repeated, numerous washings. Total recovery in lung and wash ranged

TABLE II.—*In Vitro Dose of Barbiturates in Rats and Guinea Pigs*

Barbiturates	pKa	Guinea pigs		Rats	
		Dose (γ)	Number of Lungs	Dose (γ)	Number of Lungs
Phenobarbital	7.3	100	15	150	15
Pentobarbital	8.1	100	15	50	22
Thiopental	7.6	100	19	100	19
Hexobarbital	8.2	337.5	18	337.5	25

TABLE III.—*In Vivo Dose of Barbiturates in Rats and Guinea Pigs*

Barbiturate	Guinea Pigs			Rats		
	Dose mg/Kg	Number of Animals	Induction Time (min)	Dose mg/Kg	Number of Animals	Induction Time (min)
Phenobarbital	150	18	12	125	18	12
Pentobarbital	40	18	5	40	24	5
Thiopental	40	18	3	40	20	3
Hexabarbital	125	22	2.5	125	24	2.5

between 95–105%. Total recovery from lung and from perfusate effluent in the experimental isolated lungs was in the same range.

Pentobarbital concentration was the most persistent in both species. More than 40% of the amount injected was present 60 minutes later. This concentration persisted in guinea pig lungs to the end of the experiment at 120 minutes. In rat lungs, however, in the next 40 minutes the concentration fell to 25% of the initial. In rat lungs also, the other three barbiturates showed typical half-life decay curves which were parallel to each other. In each of these instances, about 25% of the initial concentration remained at the conclusion. Thus, in this species, all four barbiturates eventually had the same residue ratio. In guinea pig lungs, hexobarbital showed a rapid rate of decline: almost all was lost in about 120 minutes. The other two barbiturates had half-life decay curves parallel to each other, parallel to those seen in the rat, and eventual residues similar to those found in the rat lungs: 25% of the initial concentration (Figure 3).

The parallel concentration declines in the lungs within each species would suggest metabolic similarity. The parallelism between the species, in the rate of decline of thiopental and phenobarbital, and the similarity of their residue ratios together with the maintenance of the level of pentobarbital, may indicate metabolic similarity between these two species. Nonetheless, relatively high concentrations of all except guinea pig hexobarbital remained for as long as 120 minutes after a single injection. This would suggest that successive injections or perfusion would increase this concentration sufficiently so that the lung might lose its inflation capability.

In vivo circulating barbiturate may be considered to be perfusing the lung. Assuming equal initial concentration of barbiturate in all tissues after intraperitoneal injection, the lungs of both species showed initial concentrations two to ten times higher than this. The *in vivo* concentration of pentobarbital in rat and guinea pig lungs remained at high levels for up to six hours, despite decreasing blood concentrations. In guinea pig lungs, the concentration fell only from 600% to 350 and in rat lungs the concentration remained at 200% for the first four hours, and then fell to 60% in the two following hours. The retention was similar to that seen *in vitro* (Figure 4). The concentration of each of the other barbiturates, in the lungs of both species, also remained high, but showed progressive declines (Figures 5–7). Enough remained after six hours, however, to have had serious effects on the *in vitro* lungs.

These findings confirmed the observation mentioned above, that after barbiturate overdosage, lung function needs to be maintained while other therapy is in progress. This must be done with positive pressure respiration, since, as was shown above, the lung loses its capability to inflate and deflate by negative pressure. The positive pressure augmentation should be continued until inhibitory lung concentrations have been reduced.

The alveoli inflate and deflate without muscle

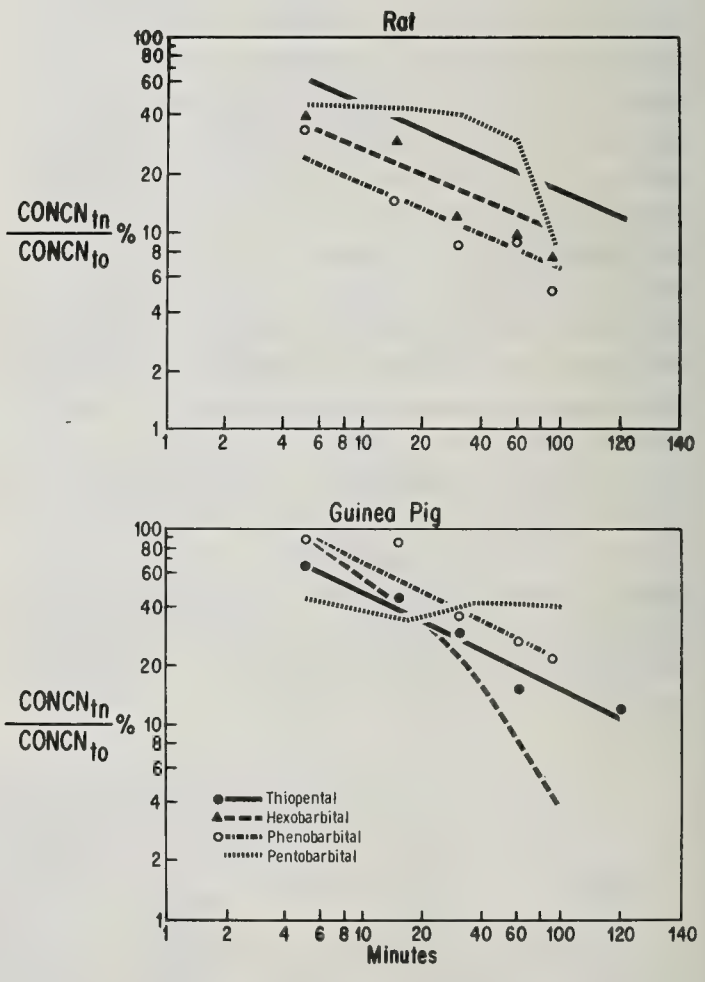


FIGURE 3.—*In vitro* rat and guinea pig lung concentrations of indicated barbiturates.

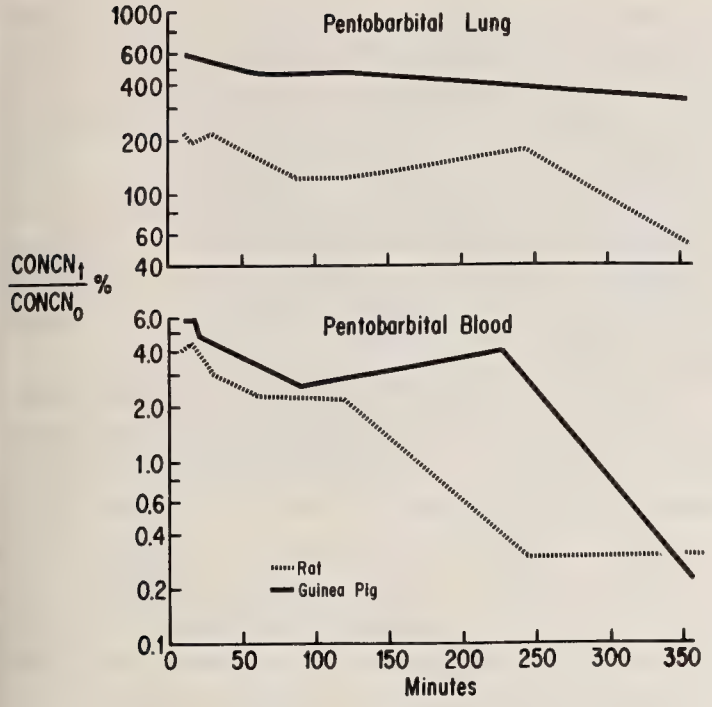


FIGURE 4.—Lung and blood concentrations of pentobarbital in rats and guinea pigs.

fibers. The experiments described above, and others detailed below, indicate that the elasticity of the alveoli may be reversibly or irreversibly modified. The nature of this modification is not known. It may be related to pulmonary surfactant, and this needs to be investigated.

The *in vitro* effects in guinea pig lungs of autonomic drugs and of histamine after pulmonary artery injection or aerosol inhalation have been reported.[1,9] Acetylcholine and carbachol reduced tidal volume or suppressed respiration completely, as did DFP, parathion and paraoxon.[7] Tidal volume restoration by atropine was minimal, but atropine pretreatment blocked the action of both the anticholinesterases and the cholinergic drugs. The tidal volume effects of these drugs were reversed by isoproterenol. Not surprisingly, epinephrine exerted a smaller antagonistic effect than did isoproterenol. The respiratory inhibition induced by serotonin, was antagonized by isoproterenol.[8] The slow development of effect and the similarity of effects by airway administration and by pulmonary artery injection would indicate that the effects were predominantly on the airways rather than the alveoli. The severe, often irreversible suppression of respiration induced by histamine was also probably predominantly an airways effect, but the large Mast cell population in the

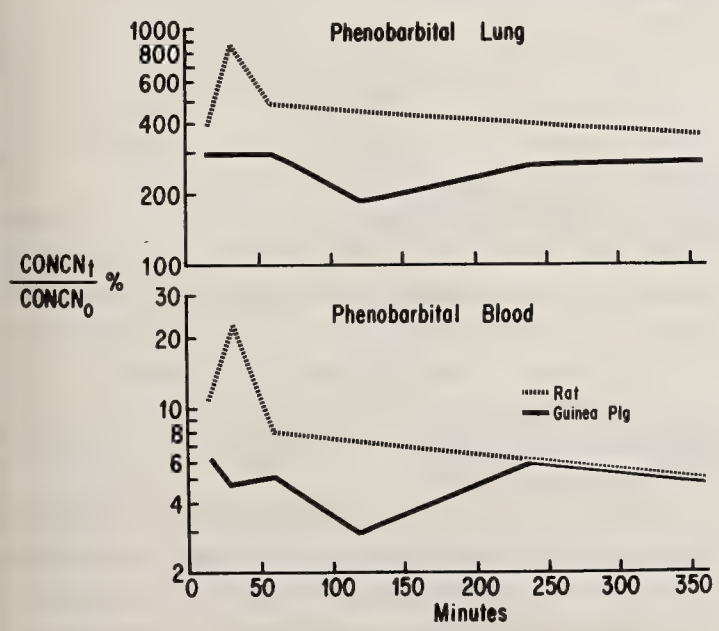


FIGURE 5.—Lung and blood concentrations of phenobarbital in rats and guinea pigs.

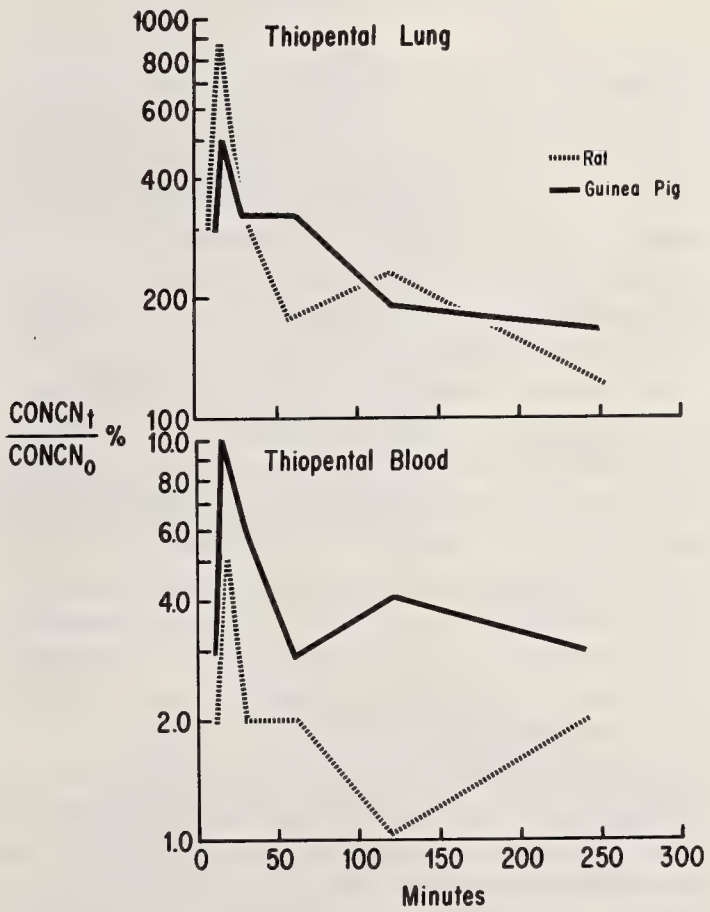


FIGURE 6.—Lung and blood concentrations of thiopental in rats and guinea pigs.

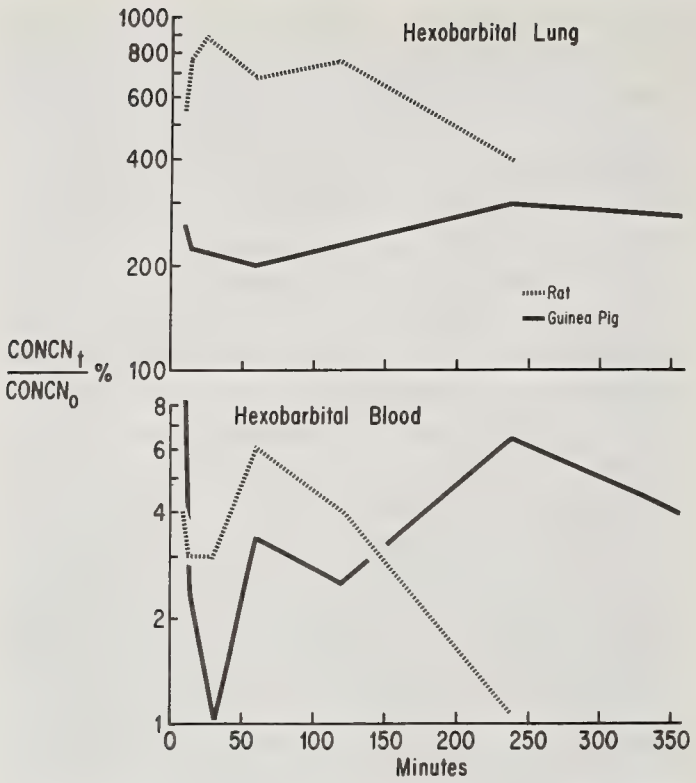


FIGURE 7.—Lung and blood concentrations of hexobarbital in rats and guinea pigs

guinea pig alveoli would indicate the possibility of significant alveolar involvement. The immediacy of the effects of 5 gammas of histamine would suggest this. Serotonin was reported to cause pulmonary vascular constriction in the guinea pig lung,[8] but the effects of the other autonomic drugs on the guinea pig pulmonary vasculature were not reported.

Rat lungs were little affected by histamine. One thousand μg induced only an irreversible 3% reduction in tidal volume which occurred five minutes after the injection. A second 1000 μg injection, 10 minutes later, reduced the tidal volume by another 3%. The delayed response indicated airway rather than alveolar effect. Each of the two injections caused a transient pulmonary vascular constriction, shown by a 15% reduction in perfusion flow rate.

Acetylcholine, 40–160 μg injected into the pulmonary arteries of rat lungs induced dose related decreases in tidal volume. These occurred immediately after injection and recovered partially or entirely within five minutes (Figure 8). Visible edema, which developed immediately upon injection, seemed to have had only minimal effects on tidal volume. The effects were blocked by prior atropine. Tidal volumes were also reduced rapidly and reversibly by 20–80 μg of isoproterenol. Propranolol blocked partially or completely. Norepinephrine, 100–400 μg, also showed dose-related rapid and reversible tidal volume reductions which could be blocked wholly or partially by dibenamine. No edema was seen with either isoproterenol or norepinephrine.

Blood vessel responses with all of these autonomic drugs were also blocked by their blocking agents, but the responses were not typical of those seen in systemic vascular responses. Acetylcholine caused dose-related vascular constriction. Perfusion flow rate was reduced as much as 50%. Isoproterenol induced vascular dilatation, or had no effect. The dilatation in one case was shown by a 50% increase in perfusion flow rate. Propranolol blocked this dilatation. Norepinephrine showed a reverse effect. Higher doses and repeated doses were less constricting than lower doses or single doses. Vascular dilatation occurred after dibenamine.

SUMMARY

In general, it is apparent that pulmonary artery injection of the various drugs into the isolated rat lung affected the alveolar bed primarily, while in the guinea pig lung the more conspicious effect was on the airways. The capability of the alveoli to inflate and deflate may be modified by barbiturates and by catecholamines, but the mechanism involved with this capability is still unknown. It may be related to pulmonary surfactant.

The barbiturates were retained in both species for prolonged periods of time in concentrations which exerted persistent inhibitory effects. This persistence may have significance in treatment of barbiturate overdosage. It may be necessary to drive the lungs with positive pressure until lung concentrations have been reduced sufficiently.

The pulmonary vascular system in the guinea pig lung has not been investigated, but in rat lungs it does not appear to respond in the same way as the systemic vasculature.

The isolated cat lung will make an interesting preparation. The absence of any connection

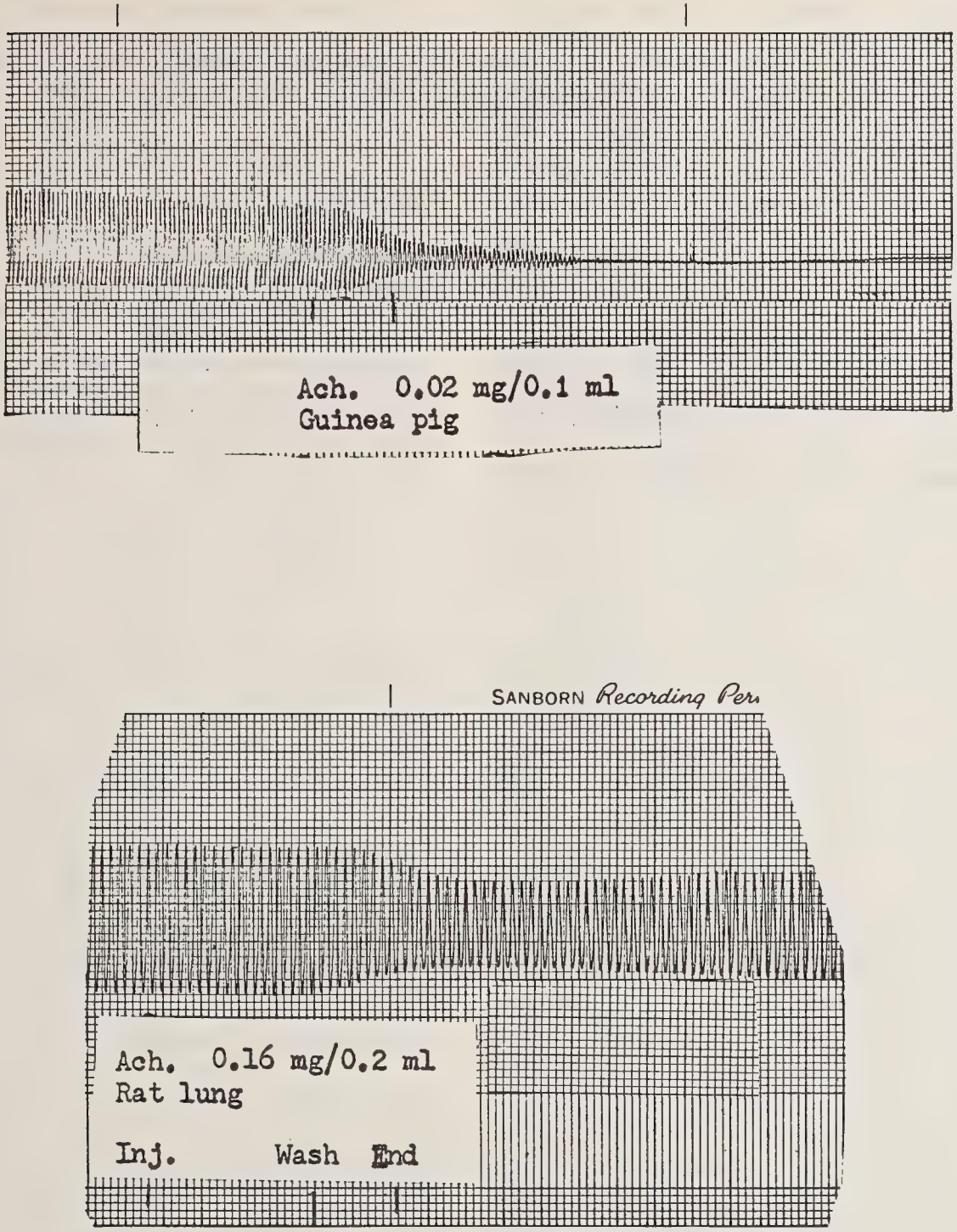


FIGURE 8.—Reversible (rat) and irreversible (guinea pig) respiratory effects of *in vitro* acetylcholine.

between pulmonary and bronchial vasculatures will make it possible to unequivocally distinguish between alveolar and bronchiolar responses, and it will permit the direct comparison between the pulmonary and systemic vasculature, by injecting the pulmonary and bronchial artery alternately.

REFERENCES

1. HEYMANS, C., and DAUTREBANDE, L. Effects of Drugs and Dust Particles on Volume of Isolated Perfused Guinea-Pig Lung. Int. Pharmacol. Meet. 1st, Stockholm, 9:151–181, 1961.
2. LAUCHE, A. Trachien, Bronchien, Lungen und Pleura. P. Cohrs, R. Jaffe und H. Meesen, Chap. B., Vol. I in *Pathologie der Laboratoriumstiere.* Springer-Verlag, 1958.

3. Camarri, E., and Marini, G. *La Circulation Bronchique*. Editions Doin, Deren & Cie., Paris, pp. 1–300, 1965.
4. McLaughlin, R. F., Jr., Tyler, W. S., and Canada, R. O. Subgross Pulmonary Anatomy of the Rabbit, Rat, and Guinea Pig, with Additional Notes on the Human Lung. Jour. Resp. Dis. 94:380–387, 1965.
5. Aviado, D. M., *The Lung Circulation*. Two Vol. Pergamon Press, pp. 1–1405, 1965.
6. Bhattacharya, B. K. A Pharmacological Study on the Effects of 5-Hydroxytryptamine and its Antagonists on the Bronchial Musculature. Arch. Int. Pharmacodyn. 103:357–369, 1956.
7. King, T. O., Poulsen, E., and Rovati, L. A. The Effects of Anticholinesterase Drugs on the Isolated Guinea Pig Lung. Arch. Int. Pharmacodyn. 110: 77–82, 1957.
8. Bhattacharya, B. K. Antispasmodic Properties of Certain Quaternary Derivatives of 2,2-Diphenyl-4-diisopropylamino-butyramide and other Compounds in the Isolated Perfused Guinea-pig Lung. Arch. Int. Pharmacodyn. 104:285–292, 1956.
9. Delaunois, A. L. A New Isolated Lung Apparatus. Arch. Int. Pharmacodyn. 148:597–606, 1964.
10. Sperling, F. Marcus, W. L., and Delaunois, A. L. Responses of Isolated, Perfused Rat and Guinea Pig Lungs to Barbiturates. Arch. Int. Pharmacodyn. 176:309–318, 1968.
11. Heaf, P. J. D., Deaths in Asthma: A Therapeutic Misadventure? Brit. Med. Bull. 26:245–247, 1970.
12. Marcus, W. L., Sperling, F., and Goldbaum, L. R. A Comparison of the *In vitro* and *In vivo* Effects of Thiopental, Hexobarbital, Pentobarbital, and Phenobarbital on Lungs of Rats and Guinea Pigs. Ph.D. Dissertation, Howard University, Washington, D. C., 1971.

THE EFFECT OF HYPOXIA ON THE PULMONARY MICROCIRCULATION

W. W. Wagner, L. P. Latham and D. R. Halsey*

The pulmonary vasopressor response to airway hypoxia has been well established. There is evidence that there is a resistance rise in both pre- and postcapillary vessels, but the effect on the pulmonary capillary bed of vasomotion at both these vascular levels is not clear. We have studied the effects of hypoxia on the pulmonary microcirculation via a thoracic window implanted in dogs. An estimation of the number of perfused capillaries was made using a grid count technique. With the spontaneously breathing dog lying on his side, studies were made of either the dependent lung (bottom of the lung) or the superior lung (top of the lung); $n = 4$ in each case. Preliminary analysis indicates a significant effect of gravity on the number of perfused capillaries: capillary grid counts on the top $= 53 \pm 7$ (SEM) versus bottom $= 153 \pm 11$ ($p < 0.01$). With isocapnic hypoxia there was a striking and consistant increase in the number of perfused capillaries (capillary recruitment) at the top of the lung: control $= 53 \pm 7$, hypoxia $= 92 \pm 9$ ($p < 0.01$). At the bottom of the lung where the capillary bed was more nearly filled to capacity, hypoxia still produced recruitment: control 153 ± 11, hypoxia 173 ± 14 ($p < 0.01$). In 12 cases cardiac output increased with hypoxia, but in 5 cases it decreased. Capillary recruitment occurred in all cases regardless of the direction of change in cardiac output. Also recruitment was independent of the magnitude of the rise in pulmonary artery pressure. Because recruitment cannot be explained by the magnitude of the increase in pulmonary artery pressure or by cardiac output changes, capillary recruitment with hypoxia is probably caused by constriction of postcapillary vessels. This pulmonary microcirculatory response to hypoxia serves to increase the surface area of the air-blood interface and therefore could be advantageous for gas exchange during airway hypoxia.

INTRODUCTION

To understand the lung as a gas exchange mechanism, it is important to define pulmonary function both in terms of gas transport from the atmosphere to the exchange surface as well as blood transport to the alveolar capillary membrane. To operate efficiently the two transport systems must interact in a balanced fashion so that blood and gas reach the same regions of the lung in proper proportions for optimal gas exchange.

It appears that one of the ways a ventilation-perfusion ($\dot{V}/\dot{Q}$) balance is maintained is by the pulmonary vasopressor response to airway hypoxia. Thus if one lung becomes hypoxic, the constricting vessels in that lung shunt the blood to the normoxic lung.[1-3] If both lungs are made hypoxic, the normal hydrostatic perfusion gradient favoring blood flow in the dependent lung is diminished,[4] i.e., blood flow is directed toward the relatively underperfused upper part of the lung. Such a mechanism probably operates on a local level, so that when a small group of alveoli becomes hypoxic, blood is shunted to adjacent alveoli to maintain a $\dot{V}/\dot{Q}$ balance within small areas of lung.[5]

It is not clear what effect general airway hypoxia would have on the pulmonary capillaries because the relative contribution to elevated resistance of the pre- and postcapillary vessels to the hypoxic pressor response is uncertain. Postcapillary constriction would be expected to cause an increase in capillary filling (recruitment and distension) by causing a retrograde pressure rise. Precapillary constriction could cause recruitment at the top and "derecruitment" at the bottom of the lung by shifting blood from the bottom to the top of the lung thereby overcoming the hydrostatic perfusion gradient, or cause general derecruitment if there is a drop in cardiac output, or cause no change in the number of perfused capillaries. One reason for these uncertainties is the difficulty in studying the pulmonary microcirculation directly. Although many ingenious, indirect techniques have been devised, they suffer from the inability to resolve the small vessels with precision *in vivo*.

* Department of Medicine, University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, Colorado 80220.

The most obvious approach for precise microvascular study is direct microscopic observation of the surface of the lung *in vivo*. Indeed this technique has seemed sufficiently promising to have attracted investigators since the seventeenth century. Unfortunately the method is associated with so many problems that few workers have been successful. The problems can be divided into three categories: (1) respiratory and cardiac movement of the surface of the lung, (2) maintaining the whole animal and the pulmonary observation field in a normal physiological condition during the experiment, and (3) extracting data once microscopic observations are feasible. All these problems have to be solved satisfactorily before meaningful studies can be made.

The purpose of this paper is to outline a technique for *in vivo* microscopy of the pulmonary circulation and to report some preliminary observations on the response of the microcirculation to airway hypoxia.

MATERIALS AND METHODS

Young, adult mongrel dogs have been used in these studies, because they have a satisfactory pulmonary vasopressor response to hypoxia, tolerate the surgery well, and have a thin visceral pleura. Anesthesia is induced by intravenous administration of sodium thiopental (15 mg/kg) and is maintained by intravenous injection of α-chloralose (60 mg/kg) dissolved in polyethelene glycol 200. Following a complete forequarter amputation, a thoracic window is implanted in the chest wall in place of a resected portion of the second rib.[6] The lung under this rib has minimal respiratory movement. The window is designed to form an airtight seal with the chest wall and permit spontaneous respiration once the pneumothorax is aspirated. The air is removed from the chest by two concentric suction manifolds that are designed to arrest all movement of the surface of the lung under the window pane.[7]

Catheters are passed via the jugular vein and femoral artery into the main pulmonary artery and descending aorta, respectively. Blood pressures are measured by Statham P 23 Db transducers energized and monitored by an Electronics for Medicine DR-8 recorder; mean pressures are obtained electrically. Cardiac outputs are determined by indocyanine green dye dilution curves via a Waters XP-302 densitometer. Arterial blood samples are collected anaerobically for pH, PCO_2, and PO_2 measurements made with appropriate electrodes (Radiometer model PHM 72). Arterial oxygen saturations are calculated from the oxyhemoglobin dissociation curve corrected for pH and temperature.

During the control period, the animal spontaneously breathes a mixture of 40 per cent O_2 in nitrogen. Airway hypoxia is produced by administration of a mixture containing 12 per cent O_2, 5 per cent CO_2, and 83 per cent N_2. An average of 10 min is allowed for the animal to equilibrate on each gas mixture before any measurements are made.

The animal is placed in one of two positions for each experiment. With the microscope located above the animal, which is lying on its side, the most superior (highest) portion of the lung is viewed. This is defined as the top of the lung; the region is in zone 2 about 15 cm above the heart.[8] In the second position, the animal lies on top of the microscope. Viewing is done from the floor upward through a hole in the table. This we define as the bottom of the lung; it is in zone 3 being about 4 cm below the heart.[8]

Observations and cinemicrographs are made with a Leitz Ultropak surface illuminating microscope. An 11X objective (N.A. 0.25), a tube factor of 1X, and a Leitz 4X projection eyepiece are used in combination with a Kodak Special Reflex Model 1 motion picture camera equipped with an Angenieux 100 mm f/2 lens to produce a film magnification of 17X. The light source is a 500 watt tungsten projector bulb controlled by a variac. The light is filtered by a pair of dichroic infrared heat reflecting filters and a Kodak Wratten #64 filter. The green illuminating beam has the characteristics of being cool and increasing the contrast of the blood to the tissue.[9] When the animal has reached steady state on the control or hypoxic gas mixture, cinemicrographs are taken at 24 frames per sec for 30 sec on Ektachrome EF film.

After the film is developed, the individual sequences are cut from the roll and viewed using

an L-W motion analysis projector. The individual sequences are spliced so as to form an endless loop and projected at a magnification of 500X onto grid paper calibrated such that each grid line is equivalent to 50μ apart. The observer traces the perfused capillaries (defined as capillaries in which blood flows, as opposed to oscillates, at any time during the sequence). Every time a traced capillary touches or crosses a grid line, it is counted. The total counts are taken as an indication of the perfused capillaries for that field. Because the same field is photographed during control and hypoxia, the grid counts provide a valid comparison of the active capillaries under each condition.

RESULTS

Successful studies were made in 8 dogs: in 4 animals the top of the lung was studied and in 4 dogs we studied the bottom of the lung. These represent about half of the total number of experiments. Most of the failures were due to the visceral pleura being too thick for satisfactory observations (generally found in older animals) or because the observation field moved too much.

The animals tolerate the procedure well. Their blood-gases are normal during the control period (see Table I). The low arterial pH is probably caused by the anesthetic. However, for the purposes of this work, the acidemia serves to accentuate the hypoxia vasopressor response.[10,11] There is no evidence of shock as indicated by blood pressures.

There is a brisk rise in mean pulmonary artery pressure (Ppa) with hypoxia: from a control of 16 torr ± 0.7 (SEM) to 23 ± 1.1 torr with hypoxia ($p < 0.01$). Cardiac output rose in 12 cases with hypoxia and fell in 5 cases (see Figure 3). Arterial PCO_2 is essentially constant during control and hypoxia (see Table I).

TABLE I.—*Response of Animals to Hypoxia*

	Control	Hypoxia	Difference
P pa (torr)	16 ± 0.7	23 ± 1.1	$p < 0.01$
Cardiac output (l/min)	1.72 ± 0.09	1.82 ± 0.09	n.s.
SaO_2 (%)	97 ± 0.3	73 ± 1.4	$p < 0.01$
PaO_2 (torr)	117 ± 3.7	51 ± 1.9	$p < 0.01$
$PaCO_2$ (torr)	39.7 ± 1.8	38.9 ± 0.9	n.s.
pH_a	7.27 ± 0.07	7.28 ± 0.01	n.s.
resp. rate (per min)	14.4 ± 1.9	41.6 ± 3.4	$p < 0.01$
Temp.—esophageal (C)	38.3 ± 0.3	38.1 ± 0.3	n.s.

Values are means ± SEM

n.s. (not significant), i.e., $p > 0.05$ determined by t-test

Examples of capillary tracings are shown in Figure 1. Under all conditions there is greater perfusion at the bottom of the lung compared with the top. Hypoxia causes obvious recruitment of new capillaries at the top of the lung. Hypoxia also causes recruitment at the bottom of the lung but the new capillaries are less obvious by visual inspection because the bed is almost completely perfused during normoxia.

The capillary grid count data are shown in Figure 2 and summarized in Table II. These are based on 27 fields photographed both during control and hypoxia: 17 from the top and 10 from the bottom. At this time only one observer has made tracings from the fields, so the results presented here must be considered preliminary.

TABLE II.—*Capillary Counts (Recruitment) During Hypoxia*

	Control	Hypoxia	Difference
Top	53 ± 7	92 ± 9	$p < 0.01$
Bottom	153 ± 11	173 ± 14	$p < 0.01$
Difference	$p < 0.01$	$p < 0.01$	

Values are means ± SEM; difference determined by t-test.

More capillaries are perfused on the bottom of the lung than on the top as indicated by grid counts: bottom = 153 ± 11 (SEM) versus top = 53 ± 7 ($p < 0.01$). The greater perfusion of the bottom of the lung is consistent with the accepted concept of a hydrostatic perfusion gradient favoring blood flow to the dependent lung.[8]

With isocapnic hypoxia there is striking capillary recruitment at the top of the lung: control = 53 ± 7, hypoxia = 92 ± 9 ($p < 0.01$). These changes are consistent in that capillary counts increase with hypoxia in every case. Even at the bottom of the lung where the capillary bed is more nearly filled to capacity, hypoxia still produces recruitment: control = 153 ± 11, hypoxia = 173 ± 14 ($p < 0.01$).

DISCUSSION

After viewing the films a number of times and studying the lung visually, we are convinced that capillary recruitment with hypoxia

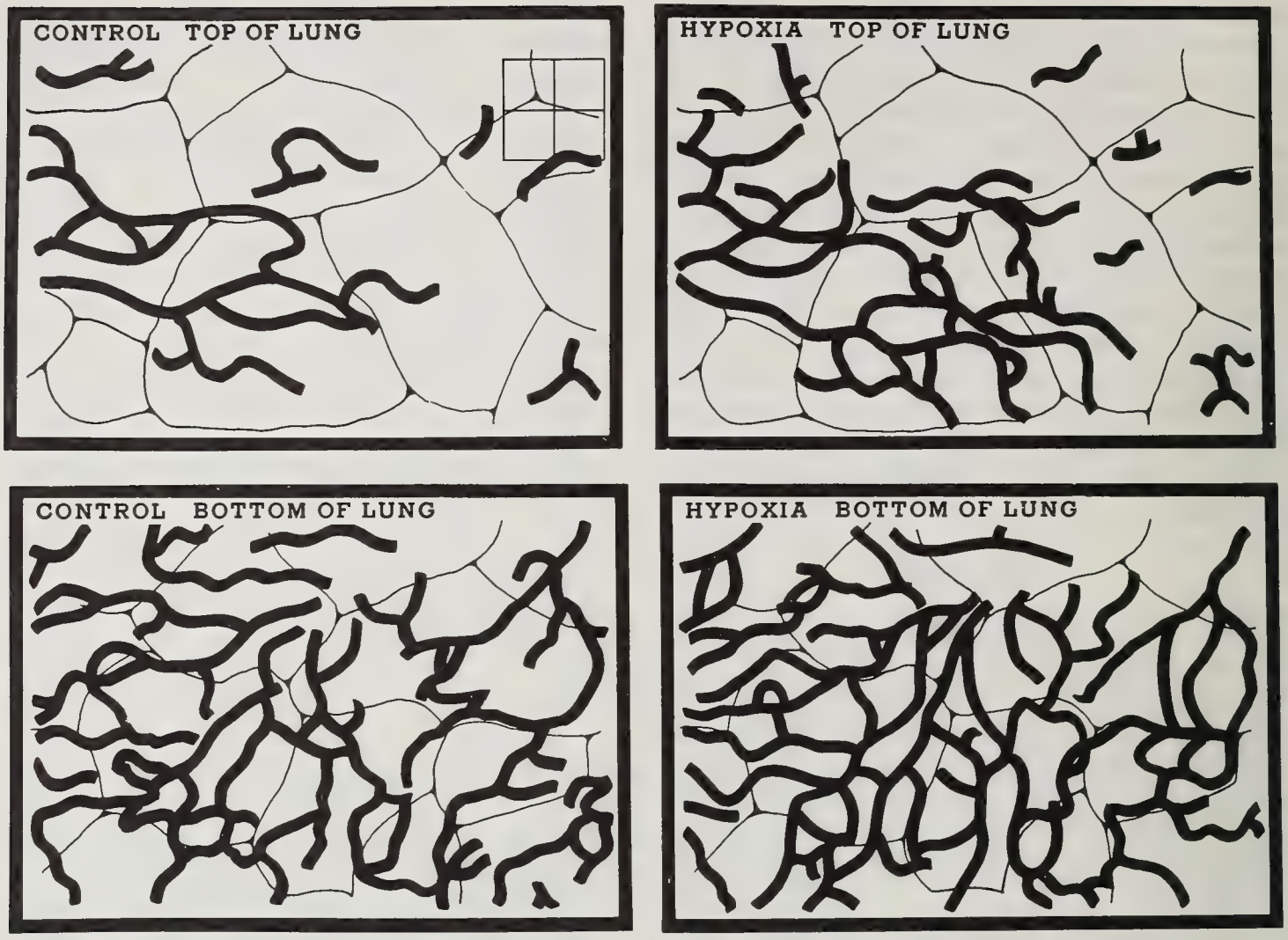


FIGURE 1.—Examples of capillary tracings on the surface of the dog lung *in vivo*. Thin lines are alveolar walls that abut with pleural surface. Heavy lines are perfused capillaries. Note greater perfusion on bottom of lung compared to top of lung under all conditions. Hypoxia causes increased capillary perfusion both on top and bottom of lung. Each square in grid (upper left) is $50\mu \times 50\mu$.

does indeed occur and that more recruitment takes place at the top of the lung than on the bottom. What is less certain is the reliability of our grid count method for estimating the magnitude of the recruitment response. The method is not objective because the observer has to judge where the capillaries are located and whether they are perfused. In many cases the perfused capillaries are obvious, but often subjective judgment is required. The reason these data must be considered preliminary is that only one observer has made a grid count analysis of the films. Undoubtably the trends indicating recruitment will be present in further analyses, but the actual numbers will almost certainly change. Careful statistical analysis will be required to determine whether the magnitude of recruitment as indicated by the grid count technique is trustworthy.

Changes in capillary diameter, erythrocyte velocity, capillary hematocrit, and red cell orientation are unmeasured. Subjectively, there do seem to be changes in these parameters during hypoxia. However, recruitment seems to be the dominant form of change in capillary volume under these conditions, especially at the top of the lung.

Given these reservations, some discussion of the data is possible in an attempt to determine the mechanism that causes recruitment with hypoxia. There are three apparent mechanisms either acting separately or in concert that could

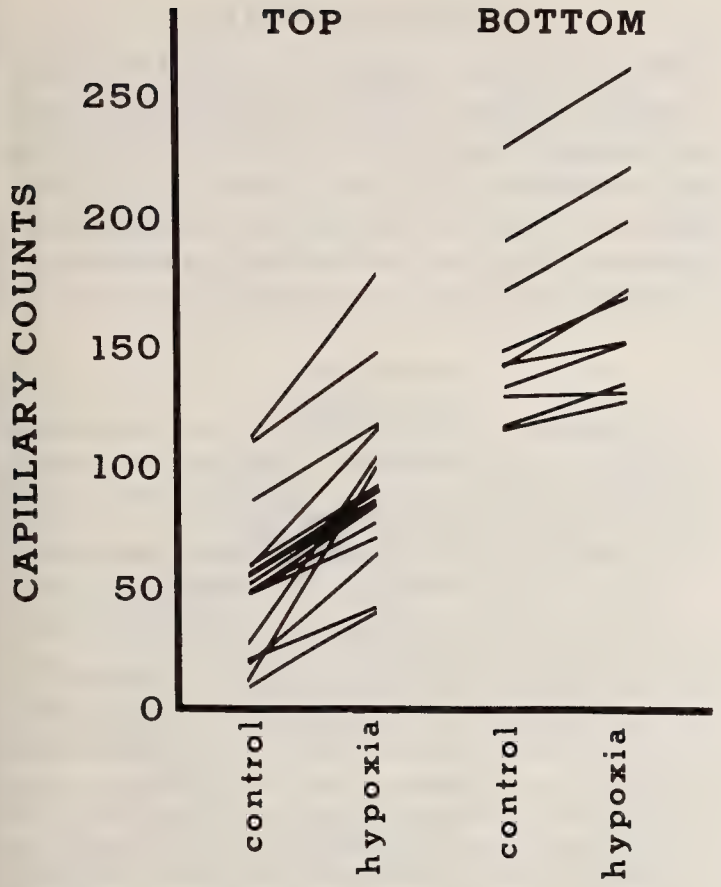


FIGURE 2.—Capillary grid count data. Each bar represents counts made on the same field during control and hypoxia.

account for the capillary recruitment we have observed with hypoxia: (1) changes in cardiac output, (2) the rise in Ppa, and (3) pulmonary venoconstriction.

Recruitment could be explained by an increase in cardiac output. This possibility has been examined by plotting the increase in capillary counts with hypoxia against the change cardiac output caused by hypoxia. The results are shown for the top of the lung in Figure 3. There is no correlation since recruitment occurred in all cases regardless of the direction of change in cardiac output with hypoxia. This conclusion is probably valid because it depends more on the trend of the capillary counts rather than on the accuracy of grid counts per se.

A second explanation is that the rise in Ppa could partially overcome the hydrostatic perfusion gradient and shift blood from the bottom to the top of the lung thereby causing recruitment at the top and derecruitment at the bottom. If this were the case, then the level of recruitment should correlate with the magnitude of the rise in Ppa. Such is not the case (see Figure 4), since recruitment is independent of the level of change in Ppa. This conclusion does depend on the accuracy of the grid counts and, therefore, should be viewed with caution. However, because there is some recruitment at the bottom of the lung with hypoxia, precapillary shifting of the blood from the bottom to the top is an unlikely explanation for recruitment.

Pulmonary venoconstriction is left as the most probable explanation. There is substantial evidence that hypoxia causes constriction of the pulmonary veins in the dog; such varied techniques as dimension transducers implanted on lobar veins,[12] pulmonary venography,[13] and various hemodynamic measurements[14-16] have been used to demonstrate this phenomenon. We conclude, therefore, that pulmonary venoconstriction is probably an important factor in causing capillary recruitment with hy-

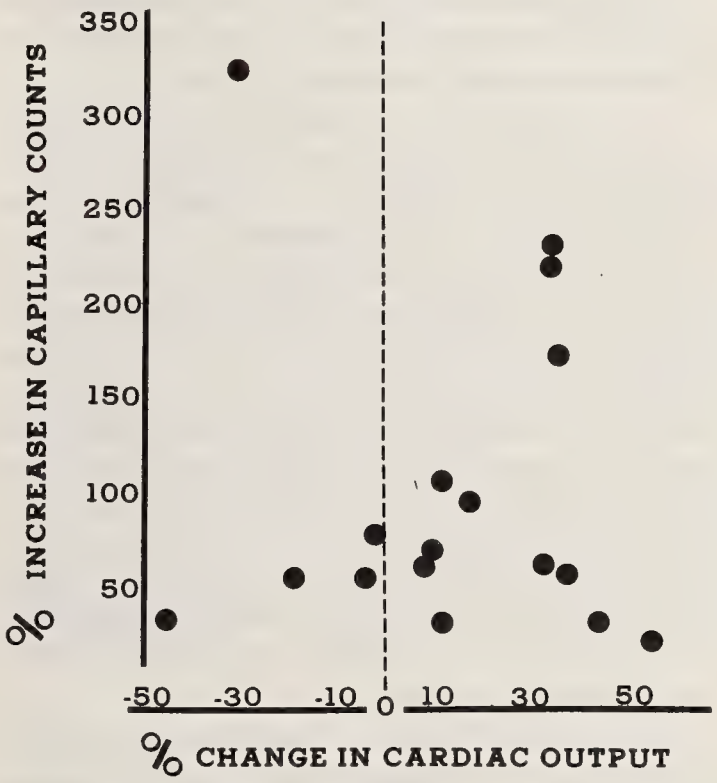


FIGURE 3.—Hypoxia produces changes in both capillary counts and cardiac output. In 5 cases cardiac output fell during hypoxia but recruitment still occurred indicating that increased capillary perfusion was not caused by cardiac output alterations. Data shown in this figure are from top of lung.

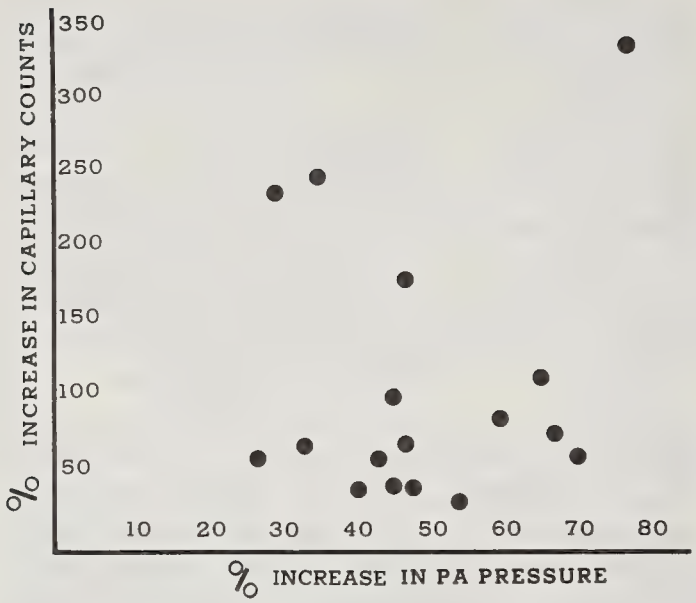


FIGURE 4.—Hypoxia produced capillary recruitment does not correlate with the magnitude of the rise in pulmonary artery pressure. Data shown in this figure are from top of lung.

poxia. The recruitment response probably serves to increase the surface area of the air-blood interface and therefore would seem to be advantageous during airway hypoxia.

As outlined in the Introduction, there are 3 problems that must be solved to make useful microscopic observations of the pulmonary circulation. The problem of movement of the surface of the lung has been solved by a combination of suction manifolds surrounding the window and by placing the window over an area of lung where respiratory movement is minimal. The motion arresting suction manifolds cause only minimal changes in the alveoli. The pulmonary microcirculation in these studies has flow patterns that we have come to accept as normal: there is no evidence of blood sludging, or hypoperfusion from emboli or other causes. The highly filtered light source does not cause detectable heat damage.

As indicated in Table I, the animals are in a reasonable physiological condition. The extensive surgical procedures do not cause shock as indicated by normal systemic arterial pressures. Both the animal as a whole and the observation field appear to be normal during these experiments.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the National Institute of Health HE 13961 and contract No. DAD417-68-C-8071 from the U.S. Army Medical R and D command. The authors are indebted to Drs. J. V. Weil, R. F. Grover, G. F. Filley, and M. Kay for encouragement and advice about this work.

REFERENCES

1. BLAKEMORE, W. S., CARLENS, E., and BJORKMAN, S. The effect of unilateral rebreathing of low oxygen gas mixture upon the pulmonary blood flow in man. Surg. Forum 5:691–696, 1955.
2. HIMMELSTEIN, A., HARRIS, P., FRITTS, H. W., and COURNAND, A. Effect of severe unilateral hypoxia on the partition of pulmonary blood flow in man. J. Thoracic Surg. 36:369–381, 1958.
3. LOPEZ-MAJANO, V., WAGNER, H. N., TWINING, R. H., TOW, D. E., and CHERNICK, V. Effect of regional hypoxia on distribution of pulmonary blood flow in man. Circulation Res. 18:550, 1966.
4. DAWSON, A. Regional pulmonary blood flow in sitting and supine man during and after acute hypoxia. J. Clin. Invest. 48:301–310, 1969.
5. KATO, M. and STAUB, N. C. Response of small pulmonary arteries to unilobar hypoxia and hypercapnia. Circulation Res. 19:426–440, 1966.
6. WAGNER, W. W. and FILLEY, G. F., Microscopic observation of the lung *in vivo*. Vascular Dis. 2:229–241, 1965.
7. WAGNER, W. W. Pulmonary microcirculatory observations *in vivo* under physiologic conditions. J. Appl. Physiol. 26:375–377, 1969.
8. WEST, J. B. *Ventilation/blood flow and gas exchange*. Blackwell, Oxford, 1967.
9. WAGNER, W. W., BRINKMAN, P. D., and FILLEY, G. F. Erythrocyte photomicrography: Contrast control by monochromatic transillumination. J. Biol. Photo. Assn. 37:156–162, 1969.
10. ENSON, Y., GIUNTINI, C., LEWIS, M. L., MORRIS, T. Q., FERRER, M. I., and HARVEY, R. M. The influence of hydrogen ion concentration and hypoxia on the pulmonary circulation. J. Clin. Invest. 43:1146–1162, 1964.
11. RUDOLPH, A. M., and YUAN, S. Response of pulmonary vasculature to hypoxia and H^+ ion concentration changes. J. Clin. Invest. 45:399–411, 1966.
12. MORGAN, B. C., CHURCH, S. C., and GUNTHEROTH, W. G. Hypoxic constriction of pulmonary artery and vein in intact dog. J. Appl. Physiol. 25:356–361, 1968.
13. QUIROZ, A. C., SANCHEZ, G., GILES, T. D., and BURCH, G. E. Angiographic demonstration of pulmonary venomotor reactions in the intact dog. Angiology 21:724–727, 1970.

14. RIVERA-ESTRADA, C., SALTZMAN, P. W., SINGER, D., and KATZ, L. N. Action of hypoxia on the pulmonary vasculature. Circulation Res. 6:10–14, 1958.
15. BERRY, W. B., MCLAUGHLIN, J. S., CLARK, W. D., and MORROW, A. G. The effects of acute hypoxia on pressure, flow, and resistance in the pulmonary vascular bed. Surgery 58:404–411, 1965.
16. FURNIVAL, C. M., LINDEN, R. J., and SNOW, H. M. The effect of hypoxia on the pulmonary veins. J. Physiol. 210:43–44, 1970.

DISCUSSION

J. R. GILLESPIE, University of California, Davis: I was wondering why you didn't consider increases in left atrial pressure as a possible explanation for recruitment?

W. WAGNER: I should have mentioned that. We measured left atrial pressure and as has been the typical finding with hypoxia, there was not an increase in left atrial pressure in these animals.

J. REEVES, University of Kentucky, Lexington: That is indeed a very beautiful movie and I'm impressed by the technical achievement of getting such pictures. It is perplexing to see the same amount of blood going through a few channels during normoxia as go through a great many channels during hypoxia. I wonder how you account for the fact that virtually all of the channels in the lungs seem to be open during hypoxia and yet the total flow is the same, or virtually the same?

W. WAGNER: One of the things that we are currently not able to do technically, is quantitate the velocity of the red cells. It may well be that although we see flow in these vessels, the velocity could be lower. I agree this is a problem. In some cases, of course, there was a change; an increase in cardiac output. The cases we saw in the film where it demonstrates recruitment the best were accompanied by increases in cardiac output. At other times, new vessels will open, but the flow is rather sluggish through them.

D. C. SAWYER, Michigan State, East Lansing: Were these untrained dogs or anesthetized dogs?

W. WAGNER: These animals are asleep throughout the procedure.

DR. SAWYER: Asleep with what? What agent did you use?

W. WAGNER: Alpha-chloralose.

R. H. REDDING, Brown University, Providence, Rhode Island: This was a very elegant demonstration visually. One thing occurred to me, and it may be very simple. I'm sure you have an answer. In measuring your cardiac output, if you used a simple oxygen consumption principle, you may well not have measured the shunting of blood which wouldn't show up in your cardiac output.

W. WAGNER: These measurements were made with green dye.

DR. REDDING: Was there much shunting? Did you notice a quick return of the dye?

W. WAGNER: We didn't look specifically at shunting, so, unfortunately, I can not give you an adequate answer.

CHAIRMAN LENFANT: Dr. Wagner, I wonder if you are aware of a paper which was published in the "Journal of Clinical Investigation" in November or December which reported experiments studying the site of the effect of hypoxia? Some very elegant experiments were reported in this paper by Murray and Blazier from San Francisco. They reached a conclusion which is quite similar to yours.

W. WAGNER: I didn't know about that paper. Since we have come up with a somewhat unorthodox explanation for our findings, I am delighted to hear of similar conclusions arrived at by independent techniques.

CINEMICROSCOPY OF THE PULMONARY MICROCIRCULATION IN SHOCK

Watts R. Webb, Stennis D. Wax, K. Kusajima, Frederick B. Parker, T. M. Kamiyama and T. Murakami*

Hemorrhagic hypotension to 40 mm Hg for 2 hours in the dog produces the full pathologic picture of congestive atelectasis with patchy atelectasis, congestion, interstitial and intraalveolar edema and hemorrhage. Pressure studies show the initial vascular response to be constriction of the small pulmonary veins.

Motion pictures have been made of the pulmonary microcirculation at powers up to 450x by using a metallurgic epi-objective microscope with dark field illumination by reflected light.

The upper portion of the lung shows early loss of capillary circulation during shock with restored flow following reinfusion, but with rapidly progressing edema, etc. However, the dependent, under side of the lung, which initially shows a much fuller vascular bed, continues with rapid flow during shock, but then has almost complete circulatory arrest on reinfusion. Stagnation, clumping of the erythrocytes and edema become more prominent and remain so.

These changes are almost completely prevented by large doses of methylprednisolone and less effectively by low molecular weight dextran.

INTRODUCTION

The pulmonary changes in irreversible hemorrhagic shock have been studied by many investigators.[1-4] Our previous work[5] confirmed that two hours of hemorrhagic shock at 40 mm of mercury followed by reinfusion of the shed blood usually produces widespread pulmonary capillary congestion. This was found to be particularly true in anemic or infected animals. It was found[6] that pulmonary reimplantation is one of the few effective methods of preventing the congestive atelectasis in the lung secondary to hemorrhagic shock, suggesting that this entity is at least in part neurogenically controlled. Subsequent work[7,8] involving multisite pressure studies on each side of the capillary bed suggested that the key response is post-capillary or small vein constriction. In summary, the initial lesion appears to be pulmonary small vein constriction with the development of a gradient between small pulmonary veins and the large pulmonary veins and left atrium. Subsequently during shock and particularly after reinfusion, an additional gradient develops across the alveolar capillary bed compatible with the interstitial edema and capillary congestion which are demonstrable microscopically.

Recently, we have been able to refine in vivo in situ microscopy viewing and photography of the pulmonary microcirculation. Direct microscopic observation of the pulmonary circulation was reported as early as 1925 by Hall using the transillumination method. In 1930, using a special Zeiss objective, Olkon[10] described the capillary circulation of the alveoli in detail, and similarly Irwin[11] published photographs of the alveolus at 500 x magnification in 1954 again using the transillumination method.

MATERIALS AND METHODS

Our apparatus consists of an inverted metallurgic epi-objective microscope using reflected incident light with dark field illumination (see Figure 1 and Figure 2). With a quartz-halogen or high pressure xenon light source it is possible to view and photograph pulmonary circulation to a depth of 1.5 mm beneath the pleura. We have been able to obtain satisfactory visualization up to 900 power and have produced bright color motion pictures at powers up to 450 with a crisp focus and a high degree of stability. A special microscope objective-tissue interface apparatus stabilizes the lung without any intrinsic damage or physiological interferences yet allowing visualization of the same alveolus and/or repeated scanning of many hundreds of adjacent alveoli for several hours. In addition, the instrumentation has been modified so that

Department of Surgery—SUNY Upstate Medical Center, Syracuse, N.Y. 13210.

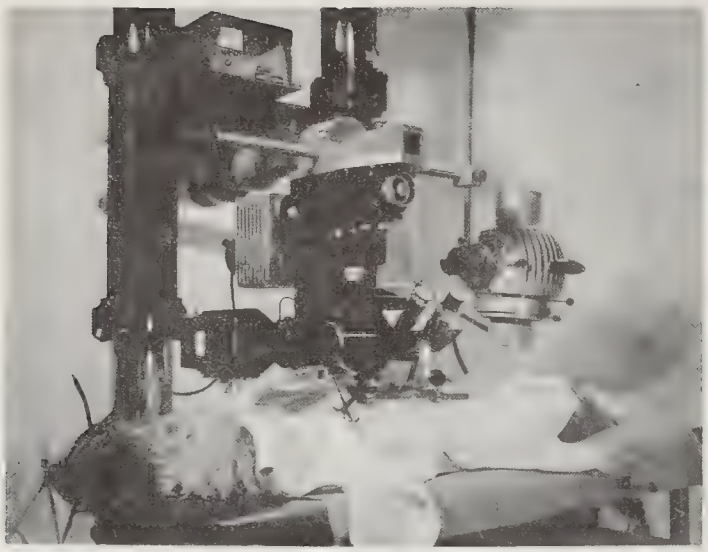


FIGURE 1.—Photograph of metallurgic microscope in position for viewing from the superior aspect of the lung. The microscope is mounted on a movable support for horizontal and vertical movement. The quartz-iodide lamp is shown behind the microscope and the xenon burner to the right. The still camera is to the left. The objective of the microscope fits against a cover slip mounted in the suction foot on the lung.

observations can be made from either the superior or inferior surface of the exposed lung. The view of the inferior, dependent surface of the lung which is subject to the higher hydrostatic force of gravity of some 10 to 15 cm of water has shown vastly different circulatory changes than those seen at the upper surface of the lung.

Healthy mongrel dogs of both sexes weighing 12–15 kilos were anesthetized lightly with pentobarbital and given three mgm heparin per kilogram. An endotracheal tube was inserted and the animals ventilated with room air with a Harvard mechanical respirator throughout the experimental period utilizing a positive end expiratory pressure of 5 cm H_2O. Tidal volume and rate were adjusted to maintain normal pH and blood gases. A femoral artery cannula was inserted for monitoring arterial pressure and another catheter placed in the femoral vein for infusions. The lung was exposed through the fifth intercostal space and the animal positioned so that the lung could be viewed through the open chest from above, or by turning the animal over, this portion of the lung became dependent and could be viewed from underneath. The animals were bled into an airless heparinized plastic bag to a mean arterial pressure of 40 mm mercury from the opposite femoral artery during a 10 minute period. At the end of two hours of observation all shed blood was reinfused over a 20 minute period. The pulmonary microcirculation was observed before and during the period of shock and for two hours after reinfusion.

OBSERVATIONS UNDER THE MICROSCOPE

Pre-shock

Arterioles. The arterioles are seen to have a pulsatile flow and to repeatedly divide in a 3-dimensional manner almost enveloping the alveoli and giving off branches at right angles which enter the capillary circulation. The amplitude of pulsation and rate of flow decreases markedly with distance from large arteriolar supply points.

Capillaries. The capillary structure allows red blood cells to pass in single file across the alveolus, each of which has from one or two to many identifiable and sometimes intermerged capillaries. The red blood cells are observed as a rapid continuous stream but the individual capillaries open and close over periods of one to five

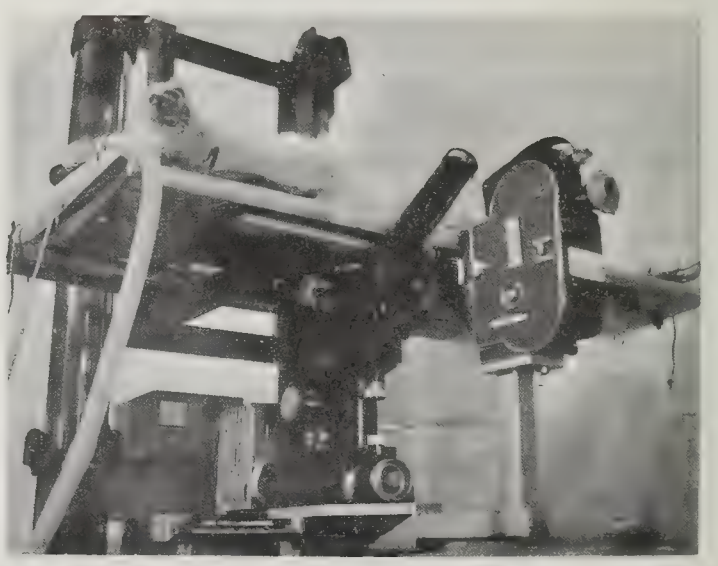


FIGURE 2.—View of microscope reversed to view the lung from underneath. The movie camera is on the right and the quartz-iodide lamp is behind the scope. The animal has simply been turned over so that the thoracotomy is dependent.

minutes as subtotal recruitment of the alveolar bed is demonstrated.

Venules. The capillaries coalesce into venules which similarly empty into wider channels. Flow through these is non-pulsatile but is quite rapid in normal circumstances. Several venules converge and the larger vein disappears into the deeper pulmonary tissues toward the pulmonary hilus.

Alveoli. The alveoli appear as large bubbles or darker structures with traversing variable capillary beds. They are roughly spherical in shape but are variable in size. The alveoli contract and expand slightly (18–20% increase in volume) with respiration. With normal respiration, flow through the capillary bed and venules around the alveoli shows little alteration, except in the alveoli most distant from the arteriolar supply, where there is some definite decrease in flow with inspiration.

Early Shock

Total volume flow appears sluggish and instead of unidirectional flow the arterial pattern shows to and fro pulsation which is accentuated by respiration. The number of plugged capillaries and venules progressively increases and only large main channels of wide venules and veins remain functional.

Late Shock

Most capillary beds are completely closed and though the capillary walls are visable, few contain any blood cells. Blood flow through the arterioles is now almost totally arrested and marked clumping of the red cells appears. Many alveoli become partially collapsed and cloudy losing their fine transparency. Hyperlucent highly refractile areas are seen in the interstitial areas suggesting interstitial edema. The venules may become dilated and very congested with packed red blood cells.

After Reinfusion

Initially there is streaming of the flow but gradually more and more of the capillaries, venules and arterioles open and progressively increase their flow. The capillary beds show extreme congestion, perivascular edema and hemorrhage. The epithelial walls are noted to become quite edematous and the venous network increases enormously in size. Many of the alveoli show increased irregularity in shape and size and many do not respond to ventilation. As the animal's general condition improves, some parts of the alveoli improve but complete recovery has not been observed in our acute experimental model even after periods up to four hours.

DEPENDENT VIEW

Normal

The capillaries, venules and arterioles are much more prominent and much wider than in the superior portion of the lung and the alveoli are less distinct. Blood flow is much faster reflecting the effect of the increased volume and flow of blood.

The entire field shows a much more vascular pattern reflecting the effects of gravity.

Early Shock

The total volume of flow does not appear to decrease as in the upper portion of the lung and circulation remains open in the arteries, veins and capillary beds.

Late Shock

The capillary beds still remain open and blood flow through the arterioles remains almost as active as during the pre-shock period. The major changes to be noted at this time are that some of the alveoli are partially collapsed and hyperlucent refractile areas are seen in the interstitial areas reflecting the development of intersititial edema. Some clumping of erythrocytes is seen but nothing to compare with the marked clumping of the superior portion of the lung.

After Reinfusion

Immediately upon reinfusion a very startling and dramatic change occurs in that circulation almost completely stops. Marked stagnation and clumping of the red cells in the arterioles and in the capillary bed develop. The capillary beds

show extreme congestion and edema and most of the alveoli appear airless. This picture remains much the same during the first hour post reinfusion but then may show gradual improvement during the next several hours.

RESPONSE TO STEROIDS

In this group of animals 30 mgs per kilogram of methyl prednisolone (solu-medrol) was administered to the animals just prior to reinfusion. All changes prior to this time had been exactly as above. Thus, this situation is quite analogous to treatment rendered in the emergency room to a patient entering in shock.

Following reinfusion, the superior portion of the lung shows a very rapid reinstitution of blood flow with far less clumping than demonstrated previously and with much less development of interstitial edema or perivascular hemorrhage.

An even more impressive change is noted in the inferior portion of the lung where flow through the hypostatic areas immediately returns to a very vigorous level following reinfusion. Flow continues and steadily improves, and there is far less alveolar collapse, interstitial edema and perivascular hemorrhage. The staining representing the margination of leukocytes, platelets and red cells along the margins of the arterioles, disappears rapidly in a fashion never demonstrated in untreated animals.

EFFECT OF LOW MOLECULAR WEIGHT DEXTRAN

In these experiments low molecular weight Dextran was administered in doses of 5 cc per kilogram at the time of reinfusion of the shed blood. In this post shock treatment, the changes previously noted in the superior and inferior portions of the lung are ameliorated though the improvement does not approach that achieved with large doses of prednisolone.

DISCUSSION

In 1945 Eaton et al.[4] observed the pulmonary changes following acute arterial blood loss and reported elevation of the peripheral venous and pulmonary arterial pressures. The pulmonary artery pressures were initially increased by intravenous infusions. Rounthwaite in 1952[12] studied variations in blood pressure in both the pulmonary artery and pulmonary veins during hemorrhagic shock and resuscitation. He concluded that both the heart and lungs play important roles though those animals with the higher pulmonary venous pressures exhibited greater degrees of congestion.

Berry, McLaughlin, Clark, and Morrow[2] in 1965, reported that acute hypoxia caused increases in pulmonary flow, arterial pressure and pulmonary arterial wedge pressure with a marked rise in the post capillary pulmonary vascular resistance. This suggested that this constriction had occurred primarily in the pulmonary veins. Keller,[7] Murakami[8] and their co-workers, using a model of hemorrhagic hypotension, found an increased resistance in the small pulmonary veins and decreased outflow of blood from the lungs leading to acute congestive lesions of the lung. Sugg, Webb and Ecker[6] demonstrated prevention of the gross and microscopic pulmonary changes by removal and reimplantation of the lung but these were not prevented by heparinization, atropine, Dibenzylene or unilateral or bilateral vagotomy. These observations suggested a neural mechanism in the pathogenesis of the lesion. Cook and Webb,[3] studying the alterations in pulmonary and systemic blood flow distribution and pressure likewise suggested that pulmonary venous constriction is a major factor in the development of acute congestive atelectasis. A most interesting related observation is that of Moss et al.[13] who showed that cerebral hypoxia with a pO_2 of 35 $\pm$ 5 mm Hg for 2 hours would produce exactly the same pulmonary pathologic changes (congestion, patchy atelectasis, interstitial and intraalveolar edema and hemorrhage) as seen following hemorrhagic hypotension of 2 hours duration. This supports our previous conclusion that the pathophysiologic changes may be mediated by the autonomic nervous system.

Our microscopic observations suggest that in early shock in the uppermost portions of the lung, blood flow almost ceases. This might in part be due to precapillary constriction, but appears to be due more likely to the lower pulmo-

nary arterial presser not elevating blood to this level above the outflow tract of the right heart. Later, perhaps as a result of the increased venous resistance, interstitial edema appears but as expected this is far greater in the dependent areas of the lung than in the superior portions. The early tremendous engorgement of the capillary beds and the progressive interstitial edema seems to be best explained by the constriction of the small pulmonary veins. Later after reinfusion the development of the capillary congestion adds alveolar capillary obstruction to the established more distal small pulmonary venous obstruction. Wilson[13] and Sherman[14] and their co-workers have similarly observed the microcirculatory changes in the lung during shock and have published findings very similar to ours. Wilson likewise found methyl prednisolone to have a protective effect for the lung in low flow states. The mechanism of this awaits full definition, but there are many effects of the steroid which might be beneficial, such as the stabilization of lysosomes, protection of the capillary endothelium, and anti histaminic effects.

SUMMARY

Continuous observations have been made during a 2 hour period of hemorrhagic hypovolemia at 40 mm Hg and following reinfusion. The pulmonary microcirculation has been photographed by use of a metallurgic epiobjective microscope utilizing dark field illumination with reflected light at powers up to 450 magnification. There are extremely significant differences in the response in the pulmonary vascular bed in the superior portion of the lung in contrast to that in the dependent areas of the lung subject to a few millimeters Hg of additional hydrostatic pressure.

REFERENCES

1. Baer, D., and Osborn, J. The post perfusion pulmonary congestion syndrome. Am. J. Clin. Path. 34:442, 1960.
2. Berry, W., McLaughlin, J., Clark, W., and Morrow, A. The effects of acute hypoxia on pressure, flow, and resistance in the pulmonary vascular bed. Surgery 58:404, 1965.
3. Cook, W. A., and Webb, W. R. Pulmonary changes in hemorrhagic shock. Surgery 64:85, 1968.
4. Eaton, R., Czebrinski, E., and Smith, J. Observation on pulmonary arterial pressure and peripheral venous pressure following arterial blood loss. J. Thoracic & Cardiovasc. Surg. 14:339, 1945.
5. Sugg, W. L., Webb, W. R., Nakae, S., Theodorides, T., Gupta, D. N., and Cook, W. A. Congestive atelectasis an experimental study. Ann. Surg. 168:234, 1968.
6. Sugg, W. L., Webb, W. R., and Ecker, R. R. Prevention of lesions of the lung secondary to hemorrhagic shock. Surg., Gyn. & Ob. 127:1005, 1968.
7. Keller, C., Schramel, R., Hyman, A., and Creech, O. The cause of acute congestive lesion of the lung. J. Thoracic & Cardiovasc. Surg. 53:743, 1967.
8. Murakami, T., Wax, S. D., and Webb, W. R. Pulmonary microcirculation in hemorrhagic shock. Surg. Forum 21:25, 1970.
9. Hall, H. A study of the pulmonary circulation by the transillumination method. Am. J. Physiol. 72:446, 1925.
10. Olkon, D. M., and Joannides, M. Capillary circulation in alveolus pulmonalis of living dog. Arch. Int. Med. 45:201, 1930.
11. Irwin, J., Burrage, W., Aimar, C., and Chesnut, R. Microscopical observation of the pulmonary arterioles, capillaries, and venules of living guinea pigs and rabbits. Anat. Rec. 119:391, 1954.
12. Roundthwaite, H., Scott, H., and Gurd, F. Changes in the pulmonary circulation during hemorrhagic shock and resuscitation. Surg. Forum 2:454, 1952.
13. Moss, G., Staunton, C., and Stein, A. A. Cerebral hypoxia as the primary event in the pathogenesis of the "Shock Lung Syndrome." Surg. Forum 22:211, 1971.
14. Wilson, J. W., Rattiff, N. D., and Hackel, D. B. The lung in hemorrhagic shock. Am. J. Path. 58:337, 1970.
15. Sherman, H., Krausner, S., Cook, W. A. Incident dark field illumination: A new method for microcirculatory study. Angiology 22:295, 1971.

DISCUSSION

J. H. Block, Cornell University, New York City, N.Y.: I'd like to congratulate Dr. Webb on what I think is a really spectacular demonstration. He has shown us some of the hemodynamics of the shock state and the normal state with the gradients between the two sides of the right circulation. Do these become abolished or improved at all with the addition of methylprednisolone?

Dr. Webb: Yes they do. We have additional

studies on this, and the gradient is much reduced with methylprednisolone. It's also much reduced in the animal with the reimplanted lung.

R. E. CLARK, Washington University, St. Louis, Missouri: A superb presentation as always, Dr. Webb. I'd like to ask you whether you've found any difference after shock with the use of hyperosmolar agents, particularly with regard to the thickness of the alveolar wall?

DR. WEBB: We have not used the very hyperosmotic solutions so I really can't answer your question. We used only the low molecular weight dextran which does not add to much, as you know.

We have tried to use lasix and things of this nature to see if this would help get rid of some pulmonary edema. It has been of little value.

DR. CLARK: Have you used heparin at all in any of these preparations?

DR. WEBB: All of these dogs were heparinized (3 mg. per kilogram), and as you can see, it does not prevent the red cell agglutination, etc.

MODELS OF CARDIOPULMONARY FUNCTION IN CALVES

J. A. Will, G. E. Bisgard, A. V. Ruiz* and R. F. Grover**

The bovine calf develops marked pulmonary hypertension when chronically exposed to high altitude. This chronic hypoxic pulmonary vasoconstriction is readily reversible by the administration of oxygen, and therefore, this striking vascular reactivity has been used by us as an *in vivo* animal screening system to test the efficacy of pulmonary vasoactive drugs in the presence and absence of chronic hypoxia.

In addition, calves fail to hyperventilate when chronically exposed to a PaO_2 of 50 mmHg. While the mechanism can not be defined at this time, certain features such as cerebrospinal fluid alkalosis, depressed central respiratory center or peripheral chemoreceptor activity, or a diminished response to acute hypoxia can apparently be ruled out. These differences between calves and man provide an interesting model for study of the comparative physiology of the regulation of respiration.

Excision of the carotid bodies in calves results in marked hypoventilation, an increase in $PaCO_2$, a decrease in PaO_2, the development of pulmonary hypertension at a moderate altitude and provides a model of chronic respiratory acidosis and cor pulmonale without the presence of airway disease.

The large accessible lung surface and the tendency to develop pulmonary hypertension make the calf useful in the study of regional ventilation and perfusion. Unlike man, the lung perfusion of the calf is apparently not entirely dependent upon gravity for its distribution and is therefore a useful model to study the relationship between ventilation and perfusion under a variety of conditions. If the differences between calves and man in their response to chronic hypoxia are recognized, bovine calves are useful as models of chronic hypoxic pulmonary hypertension, for the screening of vasoactive drugs, of hypoventilation and chronic pulmonary acidosis without airway disease, and for the study of mechanisms which relate local pulmonary perfusion to ventilation.

* Department of Veterinary Science, College of Agricultural and Life Sciences and the Cardiovascular Research Laboratory of the Department of Medicine, Medical School at the University of Wisconsin, Madison, Wisconsin.

** Cardiovascular-Pulmonary Research Laboratory, University of Colorado Medical Center, Denver, Colorado.

INTRODUCTION***

While chronic airway hypoxia causes sustained pulmonary vasoconstriction in many animals including goats[1] and llamas,[2] this response is most marked in the bovine calf. In 1963, Grover and his associates[3] reported their detailed observations of this phenomenon in Hereford steers exposed to chronic atmospheric hypoxia at high altitude. They concluded that hypoxic pulmonary vasoconstriction is the primary cause of the right heart failure syndrome occurring in cattle at high altitude and popularly known as "brisket disease." Studies by other investigators have confirmed these results.[4] This pulmonary vasoconstriction is reversible when the hypoxic stimulus is removed by oxygen inhalation or moving the animal to a lower altitude. Complete reversal of pulmonary hypertension may occur in less than two days (unpublished observations).

PULMONARY DRUG TESTING

This reversible pulmonary vasoconstriction makes the calf with hypoxic pulmonary hypertension a useful model for testing drugs with potential pulmonary vasodilator activity. Recently we took a group of calves from sea level to 3,400 m altitude at Climax, Colorado. At this altitude, PaO_2 was reduced to 50 mmHg. After two weeks, these calves had an average mean pulmonary artery pressure of 62 mmHg or 2½ times normal. The well known pulmonary vasodilator aminophylline produced transient relief of this pulmonary hypertension (Figure 1). Next we tested glucagon, since a recent report[5] indicated that this drug could lower pulmonary

*** These studies were supported by the College of Agricultural and Life Sciences, University of Wisconsin, U.S. Public Health Service Grant HE-13154, U.S.P.H.S. Special Fellowship HE 39684 and McNeil Laboratories, Inc., Fort Washington, Pennsylvania.

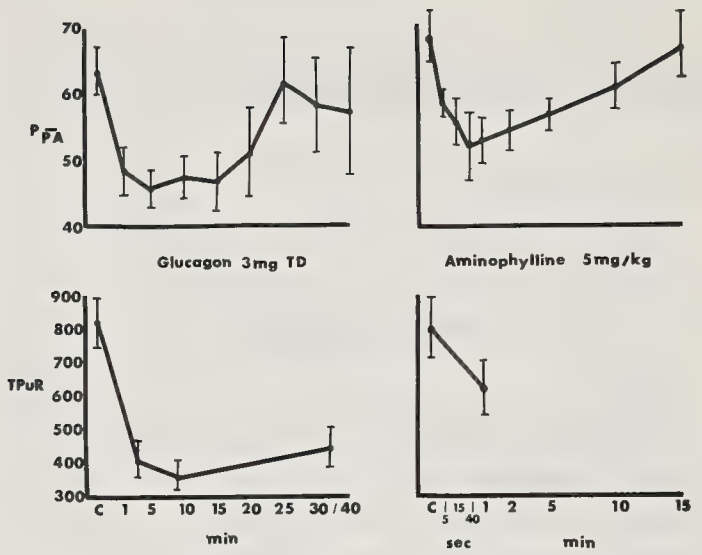


FIGURE 1.—The effects of a 3 mg total dose (TD) of glucagon as compared to aminophylline when injected IV into calves with chronic hypoxic pulmonary hypertension. n = 5.

vascular resistance. We found that glucagon produced a prolonged reduction of the high pulmonary vascular resistance in our calves (Figure 1). We have tested other vasodilators in this same animal model.

Since marked pulmonary vasoconstriction occurs in the bovine calf in response to the stimulus of airway hypoxia, this high degree of vasoreactivity can be used to test drugs which are potential pulmonary vasoconstrictors. Aminorex (Menocil®), which is pharmacologically similar to amphetamine, was suspected of having pulmonary hypertensive properties. However, when Byrne-Quinn and Grover[6] tested aminorex in the calf, pulmonary vasoconstriction did not occur.

REGIONAL DISTRIBUTION OF PULMONARY BLOOD FLOW

Pulmonary hypertension should alter the regional distribution of lung perfusion. We have recently completed one phase of a study of this phenomenon using the calf as a model.[7] Most investigators have implicated gravity as the main determinant of the distribution of pulmonary blood flow in erect man and animals. Theoretically then, the moderate increase in pulmonary arterial perfusion pressure which occurs in man and animals residing at high altitude should increase blood flow to the upper lung zone, thus improving the uniformity of ventilation-perfusion ratios and gas exchange. This would then be the adaptive "advantage" of pulmonary hypertension at high altitude. In this project we have begun to test the hypothesis that pulmonary hypertension improves gas exchange at high altitude. The calf was chosen for this study because its size provides relatively large accessible lung fields and because the calf readily develops pulmonary hypertension at altitude. Ventilation and perfusion distribution were measured using steady state breathing and infusion techniques with 133 Xenon. Probes for detection of gamma emission were placed vertically over the upper, middle, and lower zones of the right lung with the calf in a normal standing position.

In normal calves studied at sea level, perfusion per unit of lung volume (Q) was greater at the top lung than at the bottom (Figure 2). Likewise, ventilation per unit of lung volume (V) decreased from the dorsal to the ventral regions of the lung. However, these regional differences were not matched, with the result that regional V/Q decreased dorso-ventrally. The gradients are in the opposite direction to those observed in normal man, and hence do not reflect the influence of gravity observed in erect man. When these calves breathed 100% oxygen at sea level, Q increased in the ventral regions of the lung, thus reversing the initial gradient in lung perfusion. This observation implies that in the normal calf, selective pulmonary vasoconstriction in the dependent lower zones of the lung accounts for the unusual regional distribution of Q in this species. Acute hypoxia (15% O_2 or 8% O_2) did not alter the normal perfusion gradient (Figure 3).

When these calves were exposed to chronic hypoxia at 3,400 m altitude for two to four weeks, mean pulmonary artery pressure increased to 2 to 3 times normal. The regional distribution of both Q and V became more uniform, as did regional V/Q. These effects of pulmonary hypertension support our hypothesis. After four weeks at high altitude, the acute administration of gas mixtures either high or low in oxygen concentration caused little alteration

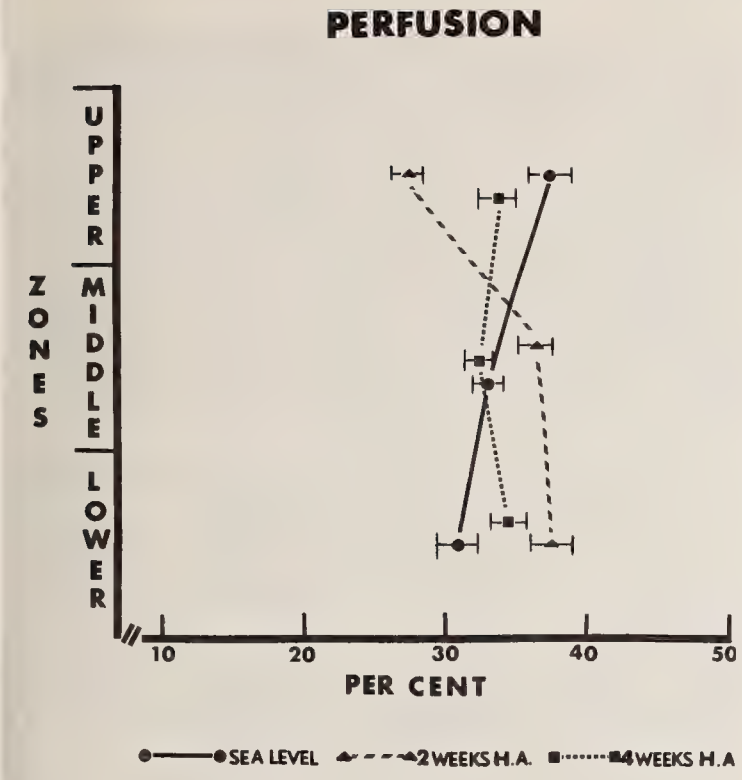
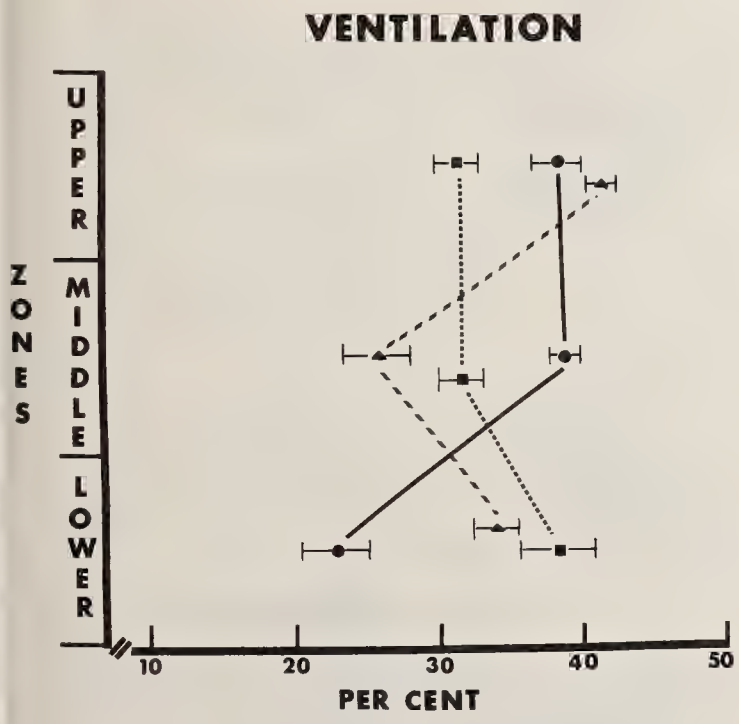


FIGURE 2.—Zonal distribution of perfusion ($\dot{Q}$) and ventilation ($\dot{V}$) at sea level (upper part) and after 2 and 4 weeks at 3,400 m altitude (lower part). Points are means ± standard error of the mean (S.E.).



in the distribution of $\dot{Q}$. Thus, while these observations indicate certain differences between the calf and man, nevertheless the calf is a very suitable model for the study of factors which modify the regional distribution of $\dot{Q}$ and $\dot{V}/\dot{Q}$ in the lung.

In normal calves, pulmonary hypertension will develop whenever the hypoxic stimulus is of sufficient magnitude. Alveolar PO_2 can be reduced solely by lowering total atmospheric pressure at high altitude, e.g., at 3,400 m where P_B is 510 mmHg. However, alveolar PO_2 will also decrease if alveolar ventilation is reduced. Bisgard and Vogel,[8] recently demonstrated that excision of carotid bodies in calves results in marked hypoventilation. The $PaCO_2$ increased by 20 mmHg while the PaO_2 decreased by 21 mmHg. When the procedure was carried out at the moderate altitude of Denver, Colorado (1,600 m), the calves became sufficiently hypoxic to develop pulmonary hypertension. However, even though calves subjected to carotid

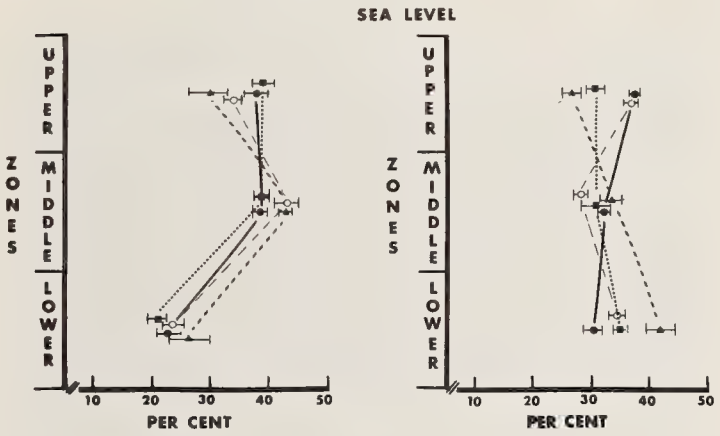
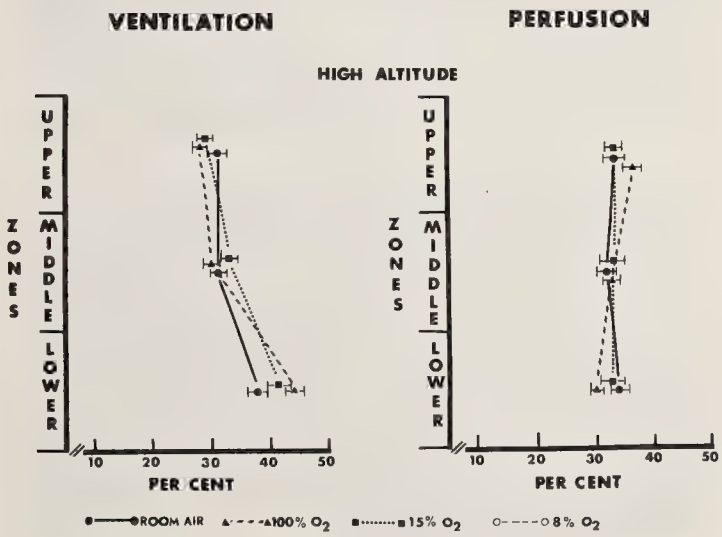


FIGURE 3.—Zonal distribution of $\dot{V}$ and $\dot{Q}$ during acute hypoxia and 100% O_2 at sea level (upper part) and 4 weeks at high altitude (lower part). Points are mean values ± S.E.



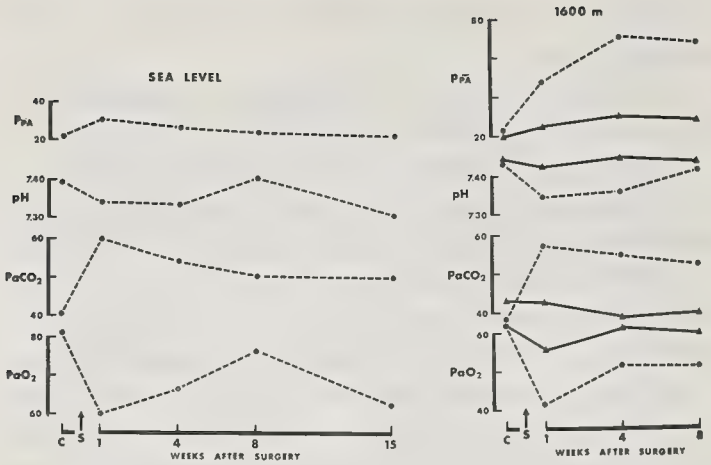


FIGURE 4.—Changes in blood gases and mean pulmonary artery pressure ($P_{\bar{P}A}$) after removal of carotid bodies (S) in calves. Control values are indicated by (C). $P_{\bar{P}A}$, PaO_2, and $PaCO_2$ are in mmHg. The solid lines represent the results from a sham operated calf.

body excision at sea level hypoventilated to the same extent, this did not lower P_AO_2 enough and they did not develop the pulmonary hypertension (Figure 4). This study showed that the carotid chemoreceptors are very important in maintaining ventilation of calves at sea level and at moderate altitudes. Thus the calf with carotid bodies removed is also useful as a model of chronic respiratory acidosis and pulmonary hypertension in the absence of airway disease.

DISCUSSION

Why does the bovine calf develop more severe pulmonary hypertension than other animals or man at high altitude? A comparison of blood gas data between man and the bovine calf at 3,400 m altitude (Table I) indicates that the arterial PCO_2 of the calf is not decreased at altitude. Grover, et al.[1] noted a transient hyperventilation and decreased $PaCO_2$ in calves at 3,800 m, but the $PaCO_2$ returned to pre-altitude values in less than 2 weeks. Hence, these steers failed to sustain hyperventilation at a PaO_2 of 45 mmHg. Later studies by Bisgard[9] demonstrated the absence of hyperventilation even during the first 24–48 hours at 3,400 m (Table II and Figure 5). PaO_2 had already reached a mean of about 50 mmHg and the mean pulmo-

TABLE I.—*Comparison of Blood Gas Data in Man and the Calf*

	Sea Level		3,400 meters	
	Man*	Calf	Man**	Calf
PaO_2, mmHg.	95	87	58	50
$P\bar{v}O_2$, mmHg.	41	35	39	33
$PaCO_2$, mmHg.	40	40	30	40
SaO_2%	97	92	90	79
$S\bar{v}O_2$%	75	57	73	46

* Data obtained from Comroe et al.[10]
** Data obtained from Grover[11]

TABLE II.—*Blood Gas and pH Values of Hereford Calves at Sea Level and 3400 Meters*

	Sea Level		3,400 meters			
			24–48 Hours		6 weeks	
PaO_2, mmHg.	87	±2*	53	±2	50	±3
$P\bar{v}O_2$, mmHg.	35	±0.5	33	±1	31	±1
$PaCO_2$, mmHg.	39	±1	41	±2	40	±1
pHa	7.39	±0.01	7.41	±0.02	7.41	±0.01

* All values are mean ± SEM in the same 6 calves.

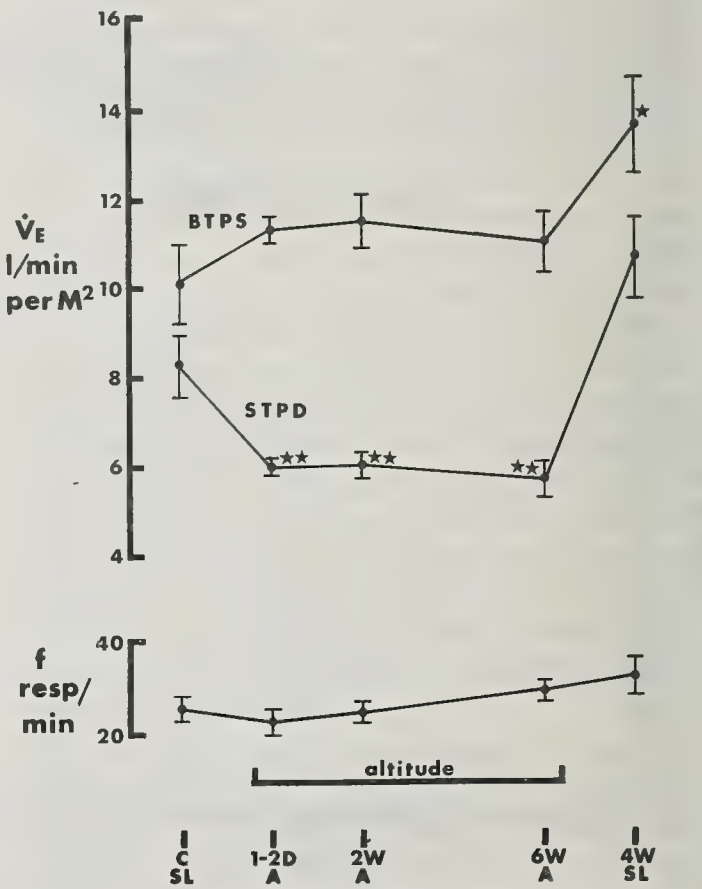


FIGURE 5.—Resting minute ventilation of Hereford calves taken to 3400 m altitude. One star indicates a statistical significance of $P < 0.05$, two stars, $P < 0.01$. f = frequency; C/SL = control, sea level; D/A and W/A indicate days and weeks at altitude or sea level.

nary artery pressure was mildly but significantly elevated when these observations were made. Therefore, in the absence of hyperventilation P_AO_2 is lower and the hypoxic stimulus is consequently greater.

From the data we now have, it is impossible to determine the mechanism for the failure of calves to hyperventilate at high altitude. We have, however, found some clues by the elimination of certain possibilities. Cerebrospinal fluid (CSF) pH was measured and found to be unchanged between the high altitude and post-altitude studies although other parameters had returned to sea level control values; therefore CSF alkalosis can apparently be excluded as the cause of relative hypoventilation. Central respiratory center and peripheral chemoreceptor activity were evaluated by the ventilatory response to the intravenous infusion of doxapram and cyanide. The responses to these drugs at altitude were similar to sea level (Figure 6) and not reduced. In addition, the response to acute hypoxia is retained at altitude and

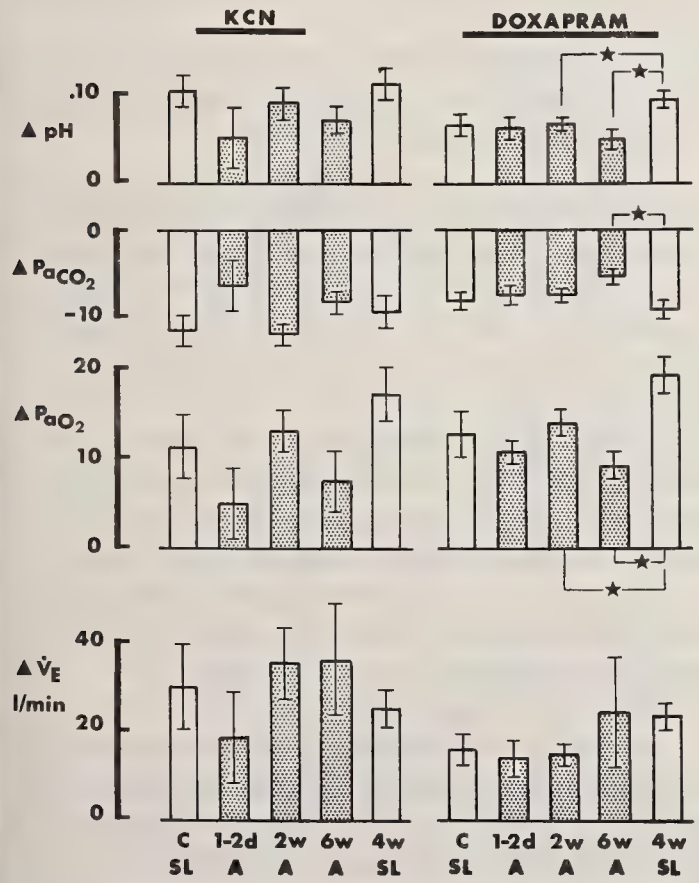


FIGURE 6.—Ventilatory responses to IV injections of KCN and doxapram.

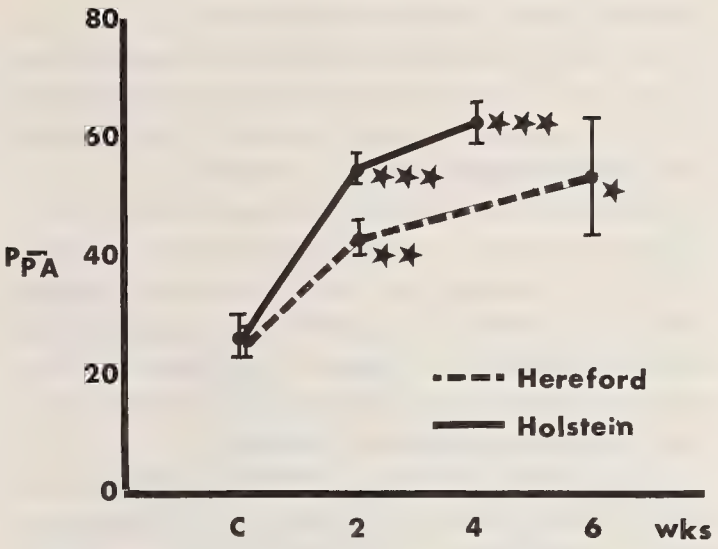


FIGURE 7.—The development of pulmonary hypertension in two different breeds of calves. The 2, 4, and 6 week levels at altitude are statistically different from the sea level control studies (C) in each case; one star $P < 0.05$, 2 stars $P < 0.01$, 3 stars $P < 0.001$. $P_{\bar{P}A}$ is shown in mmHg.

leads to the speculation that the mechanisms involved in the ventilatory responses to acute and to chronic hypoxia may be different.

We have also found that Holstein calves develop more severe pulmonary hypertension than Hereford calves when taken from sea level to 3,400 m (Figure 7). Preliminary data suggests that Holstein calves may be hypoventilating more than the Hereford calves at high altitude. Chronically hypoxic calves, then, are good models to use for the study of pulmonary hypertension in the absence of lung disease. The relative hypoventilation most certainly contributes to the development of pulmonary hypertension at high altitude.

SUMMARY

1. The bovine calf at high altitude is a good model of chronic hypoxic pulmonary hypertension that is readily reversible by the administration of oxygen or an appropriate pulmonary vasodilating drug.

2. Calves fail to hyperventilate when chronically confronted with a PaO_2 of 50 mmHg. This response is apparently not the result of cerebrospinal fluid alkalosis, depressed central respiratory center or peripheral chemoreceptor

activity, or a diminished response to acute hypoxia. The lack of hyperventilation most certainly contributes to the pulmonary hypertension and provides a good model of pulmonary hypertension without lung disease.

3. Excision of the carotid bodies in calves results in marked hypoventilation, in increase in $PaCO_2$, a decrease in PaO_2, and the development of pulmonary hypertension at a moderate altitude (1,600 m), thus providing a model of chronic respiratory acidosis and cor pulmonale in the absence of airway disease.

4. Although the regional distribution of ventilation and perfusion in the bovine lung is different from man, and perfusion apparently is not totally dependent upon gravity for its distribution, nevertheless, the calf remains a valuable animal to study the mechanisms which influence the relationship of blood flow to ventilation under a variety of conditions.

REFERENCES

1. Tucker, C. E., Will, J. A., and Grover, R. F. Pulmonary hypertension in the goat at high altitude. The Physiologist 12:378, 1969.
2. Banchero, N., Grover, R. F., and Will, J. A. High altitude-induced pulmonary hypertension in the llama (Lama glama). Amer. J. Physiol. 220:422–427, 1971.
3. Grover, R. F., Reeves, J. T., Will, D. H., and Blount, S. G., Jr. Pulmonary vasoconstriction in steers at high altitude. J. Appl. Physiol. 18:567–574, 1963.
4. Hecht, H. H., Kuida, H., Lange, R. L., Thorne, J. L., and Brown, A. M. Brisket disease II. Clinical features and hemodynamic observations in altitude-dependent right heart failure of cattle. Amer. J. Med. 32:171–183, 1962.
5. Kirsh, M. M., Kahn, D. R., Lucchesi, B., Gago, O., Dufek, J. H., Lee, R. W. S., Stutz, D., and Sloan, H. Effect of glucagon on pulmonary vascular resistance. Surg. 70:439–442, 1971.
6. Byrne-Quinn, E., and Grover, R. F. Aminorex (Menocil[R]) and amphetamine: Acute and chronic effects on pulmonary and systemic haemodynamics in the calf. Thorax 1972 (In Press).
7. Ruiz, A. V. Cardiopulmonary function in calves during acute hypoxia and hyperoxia at sea level and altitude. Ph.D. Thesis, University of Wisconsin, 1972.
8. Bisgard, G. E., and Vogel, J. H. K. Hypoventilation and pulmonary hypertension in calves after carotid body excision. J. Appl. Physiol. 31:431–437, 1971.
9. Bisgard, G. E. Ventilatory control in the Hereford calf at sea level and at 3400 m altitude. Ph.D. Thesis, University of Wisconsin, 1971.
10. Comroe, J. R., Forster, R. E., II, Dubois, A. B., Briscoe, W. A., and Carlson, E. *The Lung, Clinical Physiology and Pulmonary Function Tests.* 2nd ed., Chicago. Yearbook Medical Publishers, Inc., 1965.
11. Grover, R. F. Effects of hypoxia on ventilation and cardiac output. Ann. N.Y. Acad. Sci. 121:662–673, 1965.

DISCUSSION

J. R. Gillespie, University of California, Davis: Was the difference in the distribution and profusion in the calves about a 20 cm gradient?

Dr. Will: No, that is not centimeters, Dr. Gillespie, that's based on per cent of total lung profusion.

Dr. Gillespie: Yes, I understand that, but I meant what is your total gradient? About 20 cm?

Dr. Will: No, it's a little more than that on these calves. I would say it's more in the neighborhood of 30 cm.

Dr. Gillespie: What I'm wondering is, did you tilt the calf in any other position to see if redistribution occurred?

Dr. Will: No, we did not. However, we just finished eleven human subjects at Leadville two weeks ago, and the gradient was narrower in man than it was in the calves that we studied, so we don't feel that the gradient between the top and the bottom of the lung would be a factor in this.

Gerald Moss, Rennselaer Polytechnic Institute, Troy, N.Y.: You mentioned that the calf's arterial oxygen saturation does actually go down when he goes to altitude. Yet, I was surprised that the venous oxygen saturation didn't show a compensatory decrease also. So, it looks as if there is less of an A-V gradient.

Dr. Will: That's right.

Dr. Moss: Which means, if the animal is metabolizing normally, his cardiac output must be going up. I wonder if there's any measurement there, whether this may be contributing to the failure? I'm just wondering how much of an increase in cardiac output you have because of this hypoxemia?

DR. WILL: Well, we have an increase in cardiac output, but I don't remember the magnitude. Do you, Dr. Bisgard?

G. E. BISCARD, University of Wisconsin, Madison: I'd like to comment on that since I worked on that oxygen transport data. The A-V differences were not changed. Mixed venous PO_2 was decreased by about 2 mm of mercury. So, this suggested that we had to have a shift to the right of the dissociation curve. Plotting Van Slyke data against our arterial and venous PO_2's suggested quite a marked shift to the right of the oxygen dissociation curve. There was only a small increase in cardiac output, and there was no change in RQ and oxygen consumption.

CHAIRMAN LENFANT: If I may, I'd like to comment on that one. Do you have evidence that the bovine's blood is responsive to diphosphoglycerate?

DR. WILL: Well, it has been my understanding that they don't have much 2, 3 DPG.

CHAIRMAN: Then how come the curve shifted that much?

DR. WILL: I don't know. Dr. Alexander mentioned in his paper a little while ago that they found an increase in 2, 3 DPG. Isn't that right? But there was no shift in the curve. Is that correct?

CHAIRMAN: In addition to that, unless I missed it, I thought you were speaking of an altitude of about 3,800 meters. Is it not what you were talking about?

DR. WILL: 3,400.

CHAIRMAN: 3,400 meters. I would be quite cautious to give too much value or too much significance to the shift of the curve, if any, at that altitude.

DR. WILL: Well, we didn't mention it, as you noticed, until the discussion.

SUNG PARK, Yeshiva University, Bronx, N.Y.: Did you find any correlation between high altitudes and lactate production due, in part, to increased brain metabolism?

DR. WILL: In 1967, when we studied sedentary and training subjects at Leadville, we were unable to correlate any of the lactate data with any changes, either in the sedentary group or the trained group, at altitude. There was no correlation that we could determine at all, and we have felt that the central respiratory medullary centers were not depressed at altitudes through the use of the drug doxapram.

CHAIRMAN: I wonder if I could ask a question of Dr. Will and also perhaps Dr. Gillespie at the same time? I have been intrigued by what is meant by upper part of the lung in cows, horses or quadrupeds. Are you talking about the apical or dorsal part of the lung?

DR. WILL: In our work, we're speaking about the dorsal part of the lung when the animal is in its normal standing position, so it's not analogous to the apex in man.

CHAIRMAN: But then that brings me back to your paper, Dr. Gillespie. I believe that in the human there is clear evidence that on metastatic lesion, the blood is mostly in the upper part of the lung and that, of course, substantiates the recent observation of West on the stress that is applied to the lung due to gravitational forces. My question is, in horses do we have some special localization or typical localization of metastatic lesion?

DR. GILLESPIE: They are primarily in the apical area of the lung; so the correlation from top to bottom isn't a good one between man and the horse. In other words, the first and the most severe lesions that you'll see in a horse lung is in the apical area. This doesn't necessarily preclude the possibility that confirmation of the chest may induce different stresses on the lung, just as it does in man, but gravity, of course, would not. Fred Hoppin and I looked at the distribution of pleural pressure in the horse's lung in various positions.

CHAIRMAN: I think it's a very important issue. I wonder, did you discuss that with John West and try to conciliate your observations? He's a firm believer that one of the most significant factors in the development of metastatic lesion may be the world's gravitational forces, and apparently what you said does not really corroborate his views.

DR. GILLESPIE: Yes, John West and I have discussed at some length the distribution of pressure in the human being and in the horse and also the distribution of lesions. As far as we're concerned, his observations and ours are not reconcilable at this point.

CHAIRMAN: In conclusion, I would like to

make a plea to all of those who are doing some work on animal models, that each time you ascertain very carefully that your model is indeed comparable to the human. We might very often draw some conclusions which, in fact, are not clearly applicable to what we are attempting to do. I guess one of the spirits of this conference (which, as you know, was organized by a health agency, for humans) is perhaps to demonstrate that sometimes observations which are made in animals are not quite applicable to what we observe in man.

M. L. POVAR, Brown University, Providence, Rhode Island: I don't have a question, but just a comment. What you said just now is extremely appropriate because of some studies that are being done back at the Rhode Island Hospital in Providence. They were doing some ventilation studies in the dog and were getting very bizarre results compared with humans and were about ready to give up when we introduced the baboon. Then the results began to duplicate what they had found in man. So perhaps in this case, one animal that is normally in a horizontal position and another animal that is normally in a vertical position ventilate quite a good deal differently.

DR. WILL: It has been shown by some workers at the Mayo Clinic that the dog in the erect position is virtually identical to man.

CHAPTER IV

SURGERY AND TRANSPLANTATION

Implantation Surgery of Artificial Hearts
Experimental Cardiac Surgery in the Calf
Regional Denervation of the Canine Heart
Surgical Experience with Nonhuman Primate Animals 1963–1971
Lung Transplantation in Dogs
Experimental Aorto-Coronary Direct Revascularization in the Dog
Total Cardiopulmonary Bypass with a Membrane Oxygenator in Calves
Preservation of Organs for Transplantation
Pancreas Transplantation in Dog and Man
Left Ventricular Assist Devices in the Calf: Anesthesia, Ventilation, Antiarrhythmic Regimen, and Implantation
The Baboon in Medical Research with Particular Reference to Kidney Transplantation
Preconditioning, Implantation and Postoperative Care of the Artificial Heart Research Calf
The Use of Large Animals in Experimental Cardiovascular Surgery
Left Ventricular Hemodynamic and Dimensional Responses to Treadmill Exercises in Normal and Cardiac Denervated Dogs
Single Lung Transplantation with Immediate Ligation of the Opposite Pulmonary Artery in the Dog: A Model for Assessing the Functional Adequacy of Transplanted Lungs

IMPLANTATION SURGERY OF ARTIFICIAL HEARTS

T. Akutsu, H. Takagi and H. Takano*

This paper describes surgical techniques for implantation of various artificial hearts in calves. Artificial hearts developed in our laboratory involve bypass-type heart assist device (B-HAD), series-type heart assist device (S-HAD), and total artificial heart (TAH).

Animals were not fed for one or two days prior to surgery. Anesthesia was induced with short-acting barbituate and maintained with Fluothane. For implantation of all three types of artificial hearts the chest was opened through the fifth intercostal space. During surgery the animal was connected to a Bird respirator. The B-HAD was connected first to the descending aorta by end-to-side anastomosis of the outlet connector, and then to the left atrium. For implantation of the S-HAD and TAH, extracorporeal circulation was used. Because of particular anatomy of the ascending aorta and the aortic arch in calves, two arterial cannulas were required to perfuse the upper and lower parts of the body. Venous blood was also drained with two cannulas. One was inserted into the inferior vena cava through the right atrial appendage, and the other into the superior vena cava from the right jugular vein. During implantation of the S-HAD, the coronary arteries were perfused. The inlet cuff of the S-HAD was connected to the ascending aorta by end-to-end anastomosis, and the distal cut end of the ascending aorta was closed. The TAH was connected to the natural counterparts in the order of right atrium, left atrium, aorta, and pulmonary artery. Pumping with each device was initiated after the residual air was completely removed. The chest was closed in routine fashion. Postoperatively, antibiotics were administered routinely for about a week.

INTRODUCTION

Differences in surgical procedures in large animals from those in patients are mainly due to differences in anatomy. There are no big differences in responses to anesthetics and other drugs which are used during surgery and immediate postoperative course. This paper describes preoperative care, implantation techniques for various types of artificial hearts, and postoperative care in dogs, sheep, and calves. The devices implanted were bypass-type left heart assist device (B-LHAD), series type left heart assist device (S-LHAD), and total artificial heart (TAH). Dogs weighing about 20 kg were used only in the early stage in the series-type heart assist device experiments. The sheep and calves used weighed from 70 to 90 kg.

* Department of Surgery University of Mississippi Medical Center Jackson, Mississippi

PREOPERATIVE CARE

Experimental animals were not fed for at least one day before surgery. Ruminants, particularly sheep, were not fed for two days being allowed to take only water. When ruminants were not being fed grass or hay but were being fed pellet or mixed feed, they were used without preoperative fast since stomach irrigation was performed without much difficulty. Usually preoperative administration of antibiotics was not applied.

ANESTHESIA

All dogs were anesthetized by slow intravenous administration of Nembutal (pentobarbital, 25 mg/kg). Additional doses, ranging from 5 to 10 mg, were given if necessary.

In sheep and calves, 0.4 mg of atropine sulfate was given intravenously about 30 minutes prior to induction of anesthesia to inhibit salivation. Since it was reported[1] that these animals were sensitive to long-acting barbiturates and that their agents produced prolonged respiratory depression, short-acting Surital (sodium thiamylal) was used for induction of anesthesia in doses barely sufficient to allow endotracheal intubation (30 ml of 2.5% solution).

Intubation was performed after the animals were fixed on the operative table in supine position. For intubation of sheep and calves, tools



FIGURE 1.—Instruments necessary for intubation include an endotracheal tube, with a syringe attached for inflation of the cuff, a plastic pipe for guide of the endotracheal tube, a long, thin plastic guide tube, a flashlight, and a forceps.

developed in our laboratory (Figures 1 and 2) were used. First a plexiglass pipe (3.8 cm in diameter, and 35 cm long) was inserted. About 2/5 of its tip was cut obliquely and its end was slightly bent, enabling us to slip it under the epiglottis. Since the pipe is transparent, an ordinary flashlight held outside the pipe could illuminate the epiglottic area sufficiently. One end of a thin plastic guide line was then slipped into the trachea through the pipe. While holding it, a French 42 endotracheal tube was slipped over the other end of the guide line and



FIGURE 2.—Endotracheal tube, plastic pipe, and plastic guide line were put together, and the cuff was inflated, simulating the way of intubation.

inserted into the trachea. While holding the tube in place, the guide line was removed and the cuff on the tube was inflated. The plexiglass pipe was removed, and a short piece of another plexiglass pipe was slipped in to protect the endotracheal tube from being chewed. A thick polyvinyl chloride tube was inserted into the stomach to prevent air accumulation and also to drain out any stomach content which usually existed after one day fast.

The endotracheal tube was connected to a bird Mark 8 respirator in dogs and sheep, and to a bird Mark 4 anesthesia respirator in calves. In order to maintain anesthesia, 1.0% Fluothane and 99% oxygen was first introduced into the circuit via a Fluotec vaporizer. Anesthesia was then maintained as light as possible using 0.3 to 0.5% concentration of Fluothane most of the time. If the sheep or calves moved or tried to breathe spontaneously, a muscle relaxant such as succinylcholine chloride was given rather than increasing the amount of Fluothane. The blood pressure, being very sensitive to Fluothane, is a good indicator of the depth of anesthesia. The noninflammability of Fluothane allowed us to use electrocautery.

IMPLANTATION OF B-LHAD

The chest was entered through the fifth intercostal space. The inlet side conduit, which was provided with a Dacron cuff at one end, was connected first to the descending aorta by an end-to-side anastomosis. Then one milligram per kilogram of body weight of heparin was intravenously administered to prevent subsequent blood clotting in the cuff. In the beginning, connection of the inlet side conduit, which was also provided with a Dacron cuff, was anastomosed to the left atrium in the same way as the outlet side. In some cases, however, air was sucked in through the anastomosic line during expansion of the pumping chamber. A new technique developed later to prevent such leaking has three steps (Figure 3) : 1) anastomosis of a Dacron cuff alone to the left atrium, 2) insertion of the conduit, which has a stainless steel ring covered with Dacron velour, into the prefixed Dacron cuff to the level of the anastomosic line, and 3) connection of the Dacron cuff

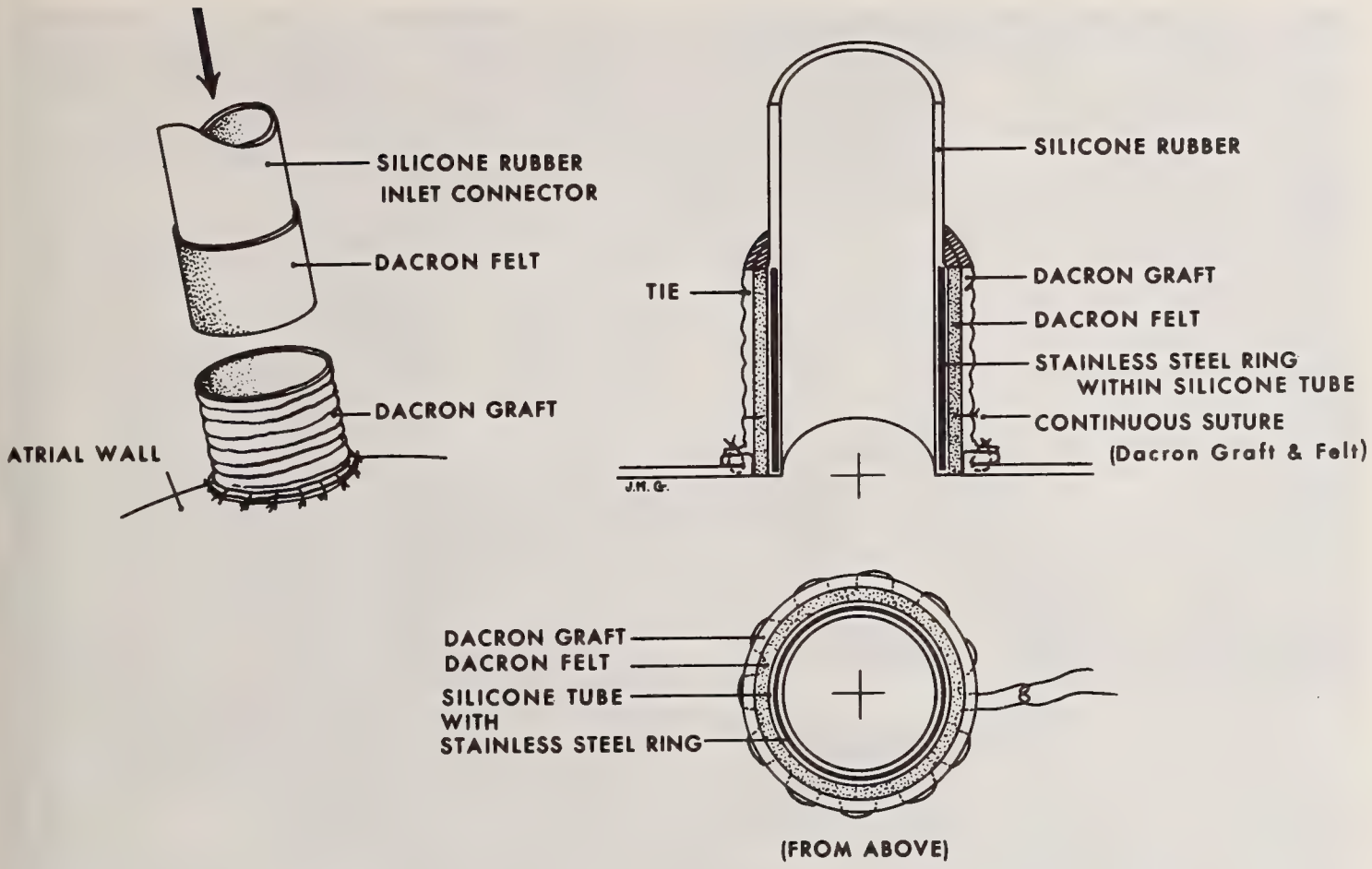


FIGURE 3.—Techniques of connection of the inlet cuff to the left atrium in the B-LHAD implantation. Series of of anastomosis starts from upper left proceeding to upper right and lower right.

and the conduit by a ligature on the distal side and by a continuous suture on the proximal side.

Upon completion of two anastomoses, the conduits were connected to the peristaltic by-pass-type heart assist device using a stainless steel ring coated completely with Silastic. After the chest was closed in routine fashion, the device was carefully filled with heparinized normal saline, and fixed on the left chest wall (Figure 4).

IMPLANTATION OF S-LHAD

The chest was opened through the fifth intercostal space both in dogs and calves. The outlet side which was provided with a Dacron graft was first connected to the descending aorta by an end-to-side anastomosis. Connection of the inlet side differed in dogs and calves.

Dogs

In dogs the inlet side of Dacron graft was connected to the ascending aorta originally by an end-to-side anastomosis. Then the ascending aorta was ligated or dissected and closed between the anastomosis and the brachiocephalic artery. Later, an end-to-end anastomosis to the ascending aorta was found to be hemodynamically preferable, and a new technique for quick connection without using a pump-oxygenator was developed (Figure 5). The Dacron cuff on the inflow side was everted over a metal adaptor ring which was split into 2 parts. After the venae cavae were temporarily occluded, a clamp was applied to the ascending aorta close to the brachiocephalic artery. After cutting the ascending aorta between the clamp and the aortic valve, the prepared cuff of the device was inserted into the proximal end of the ascending aorta and held in place with a ligature applied to the aorta against the adaptor ring. The

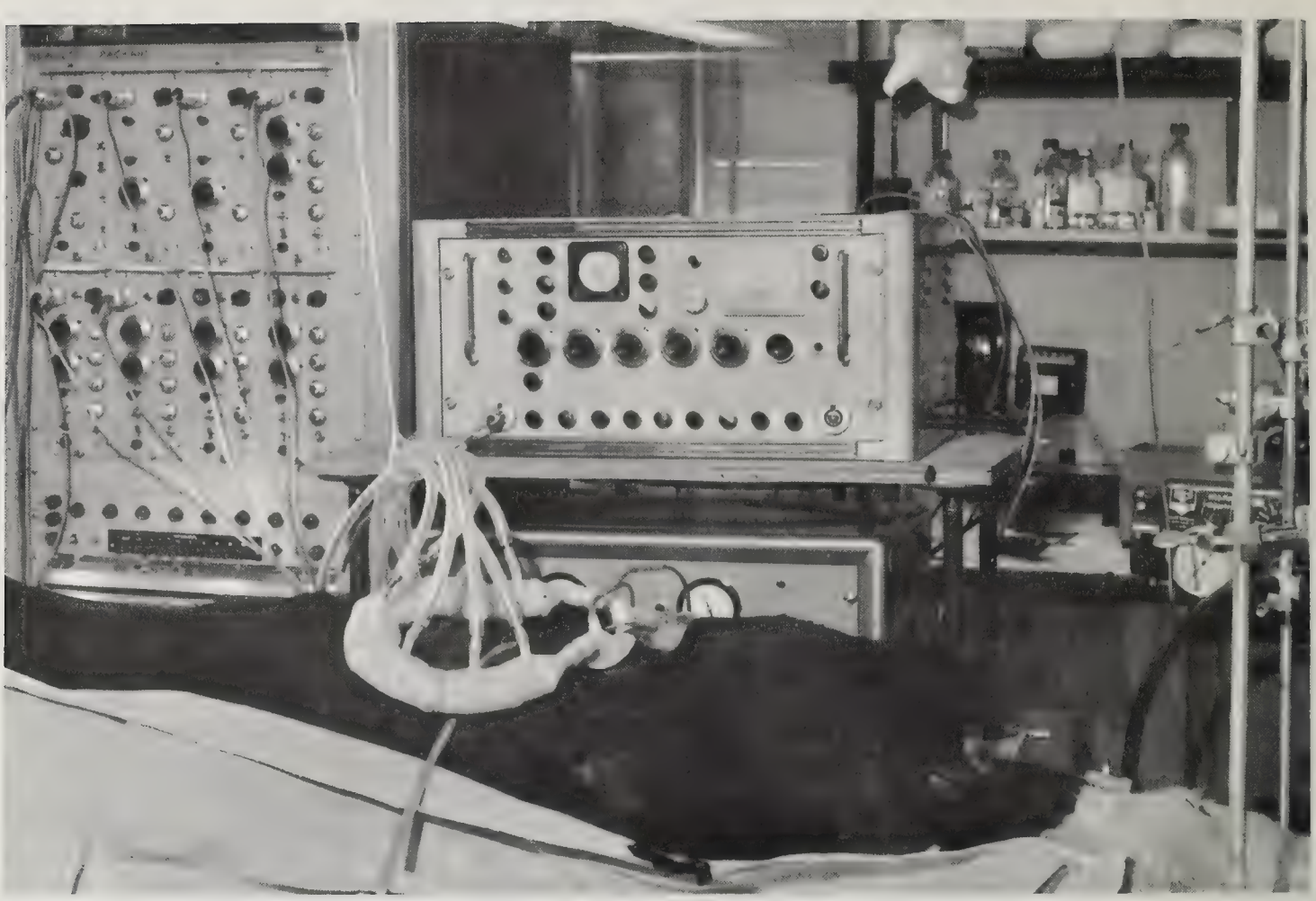


FIGURE 4.—Showing the B-LHAD fixed the chest wall. Six tubes attached to the device are compressed air lines connected to the control-driving system back of the calf. The calf's head is on the right.

clamp was then removed restoring normal circulation, since the distal anastomosis had already been completed. The temporary occlusion of the venae cavae was released. Air was removed from the unit through a needle inserted at the highest point of the device which was on the inlet side adjacent to the pumping chamber. A continuous silk suture was placed around the aorta through the Dacron cuff at the edge of the adaptor ring. When suturing was completed, the ring was split and removed. The distal end of the ascending aorta was closed by a continuous suture.

Calves

In calves, extracorporeal circulation was required for the following reason: the brachiocephalic artery is the only artery branching straight off from the ascending aorta. No other branch comes off from the aortic arch. Since the ascending aorta is extremely short, there is no room to apply the quick connection technique developed in dog experiments. In order to obtain sufficient working room around the ascending aorta, the brachiocephalic artery and the aortic arch were dissected and occluded temporarily during connection of the inlet side. This procedure required two arterial lines to perfuse each upper and lower part of the body (Figure 6). One cannula was inserted into the internal thoracic artery, and a side tube attached distal to the outlet valve was used to perfuse the lower part of the body via the descending aorta. Heparin (3 mg/kg of body weight) was administered intravenously prior to cannulation. For venous drainage one thick cannula (ID5/8″) was inserted into the right atrium through the atrial appendage. A vent cannula (ID 1/4″) was inserted into the left ventricle through the left atrial appendage.

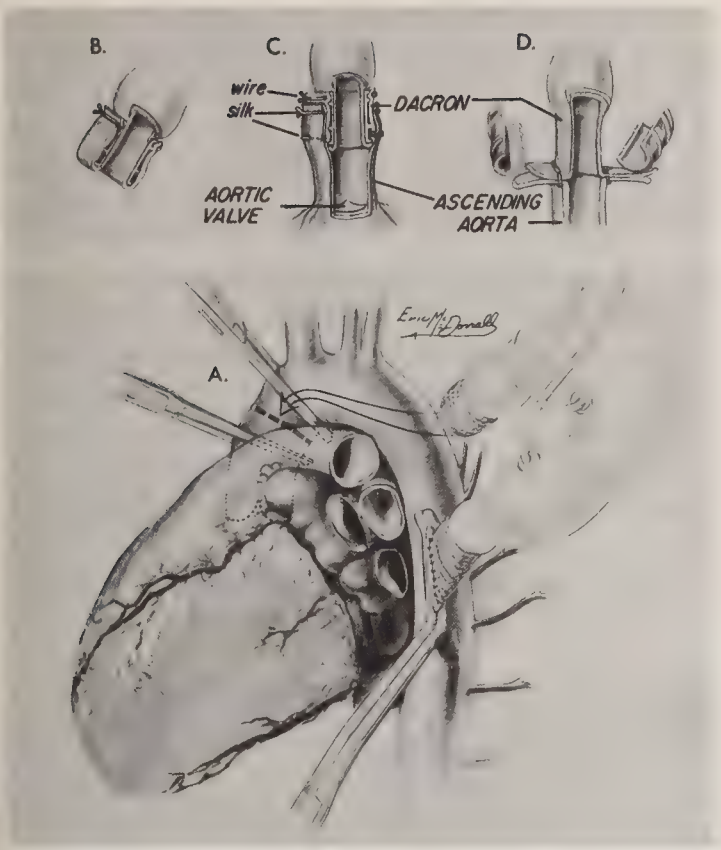


FIGURE 5.—Showing quick connection techniques of the inlet cuff of S-LHAD, which eliminates extracorporeal circulation. B: Prepared inlet side cuff with the Dacron cuff end everted over a split metal ring. C: Prepared inlet side slipped into the proximal bisected end of the ascending aorta, and a continuous suture placed around between the aortic wall and the Dacron cuff. D: Continuous suture completed, and a wire for temporary fixation of the everted cuff, a silk tie for temporary fixation of the aortic wall over the ring, and the split metal ring removed. Anastomosis completed.

After the outlet side anastomosis had been completed and the outlet conduit was clamped between a side tube and the outlet valve, extracorporeal circulation was started using a Travenol bubble oxygenator and three rotary pumps. Following bisection of the ascending aorta, French 14 and 8 cannulas were inserted each into the left and right coronary arteries. Flow for the coronary perfusion was maintained around 500 ml/min. Upon completion of end-to-end anastomosis between the inlet cuff and the proximal end of the bisected ascending aorta, residual air was replaced with heparinized normal saline through a side tube attached to the inlet side conduit, and the clamp placed between the outlet valve and the outlet side side-tube was removed, thus restoring backward flow into the device from the pump-oxygenator for coronary perfusion. When the device with a leaking leaflet outlet valve was employed, coronary perfusion was continued after implantation was completed. Because immediately after the connection was completed the heartbeat was usually not strong enough to provide sufficient coronary flow, and also the blood flowing back into the device and ascending aorta from the pump-oxygenator through a small common window at the bottom of the leaking valve was not sufficient to maintain the coronary flow. This precaution, however, was not necessary when the device with an air-driven valve was used. When the heartbeat became strong, the decompression cannula was removed and the assist device was activated following cessation of the extracorporeal circulation. The distal end of the bisected ascending

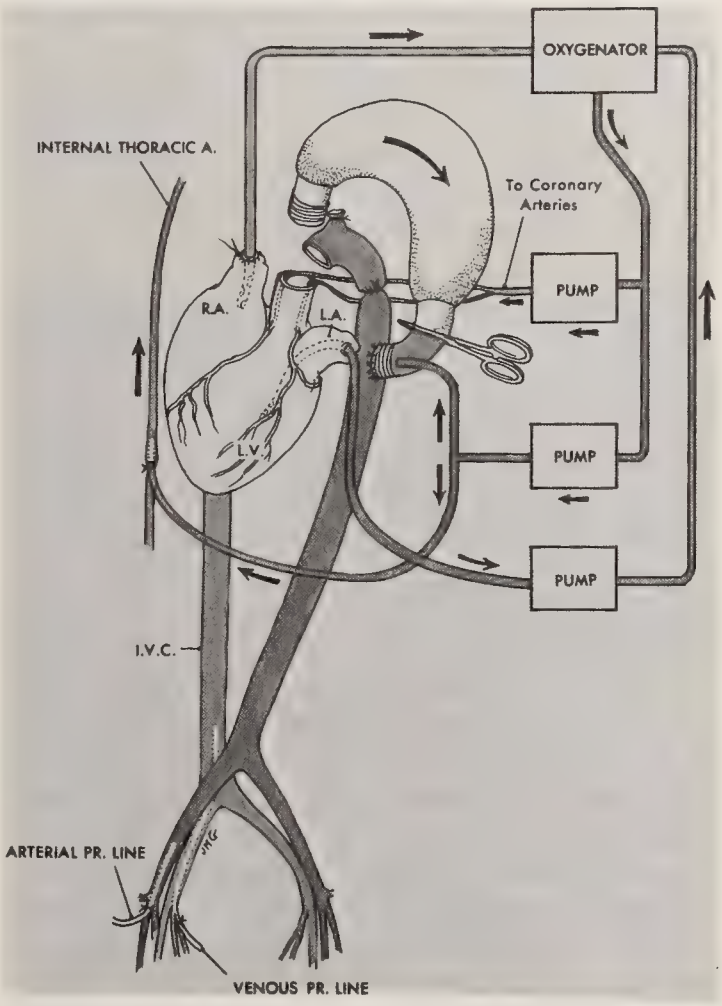


FIGURE 6.—Circuit of extracorporeal circulation during implantation of S-LHAD.

aorta was closed by a continuous suture, and the chest was closed in routine fashion.

INSERTION OF TAH

Since the mediastinum of both sheep and calves is extremely thin, sternal splitting incisions do not offer a special advantage in keeping the pleural cavities closed. Opening the chest through the fifth intercostal provides a sufficiently large operative field. In order to obtain sufficient room in the area of the short ascending aorta, as in implantation of the S-LHAD, the brachiocephalic artery and the aortic arch were dissected free to be occluded during implantation. One arterial cannula was inserted into the right internal thoracic artery and the other into the descending thoracic aorta. For venous drainage, one cannula was inserted into the supperior vena cava through the left jugular vein, and the other into the inferior vena cava through the right atrial appendage (Figure 7). Since both venae cavae are dissected inside the pericardial sac, the right chest cavity was not opened. This apparently helped keep the right lung well-inflated and prevented decreased oxygenation immediately after the artificial heart started pumping.

After occlusion of all blood flow into and out of the heart, the heart was removed, starting first with dissection of the aorta and the pulmonary artery as close to the ventricle as possible, then the ventricles. The present TAH in use consists of an entirely separate right and left aggregate. In preparing the atrial edges for separate connection of each atrium, two points were kept in mind to facilitate secure and easy connection of the atria. (1) The interatrial septum should be split sufficiently, starting from the middle of the non-coronary aortic valve cusp to give a sufficient length of the atrial edge on both sides (Figure 8). (2) The coronary sinus should be sufficiently freed close to the sinus opening and cut between two ligatures. After these procedures, connection of the prosthetic heart was done in the following order: right atrium, left atrium, aorta and pulmonary artery. When the connection was made by a single purse-string suture placed around the atrial edge over the ring embedded in the artificial atrium, bleeding was a problem. A second purse-string suture was absolutely necessary to catch small vessels arising from the bottom of the atria and opening into the fat in the atrioventricular grooves. When the atria were connected by a continuous suture, the suture was started from the caudal end of the atrial septum and continued around the right atrium clockwise, through the septum, and around the left atrium counter-clockwise to the cranial end of the septum. Immediately after the completion of the left atrial connection, the side tube attached to the left atrium was connected to the suction line to drain the bronchial return into the oxygenator.

After completion of connection, residual air was removed. For this purpose each of the four

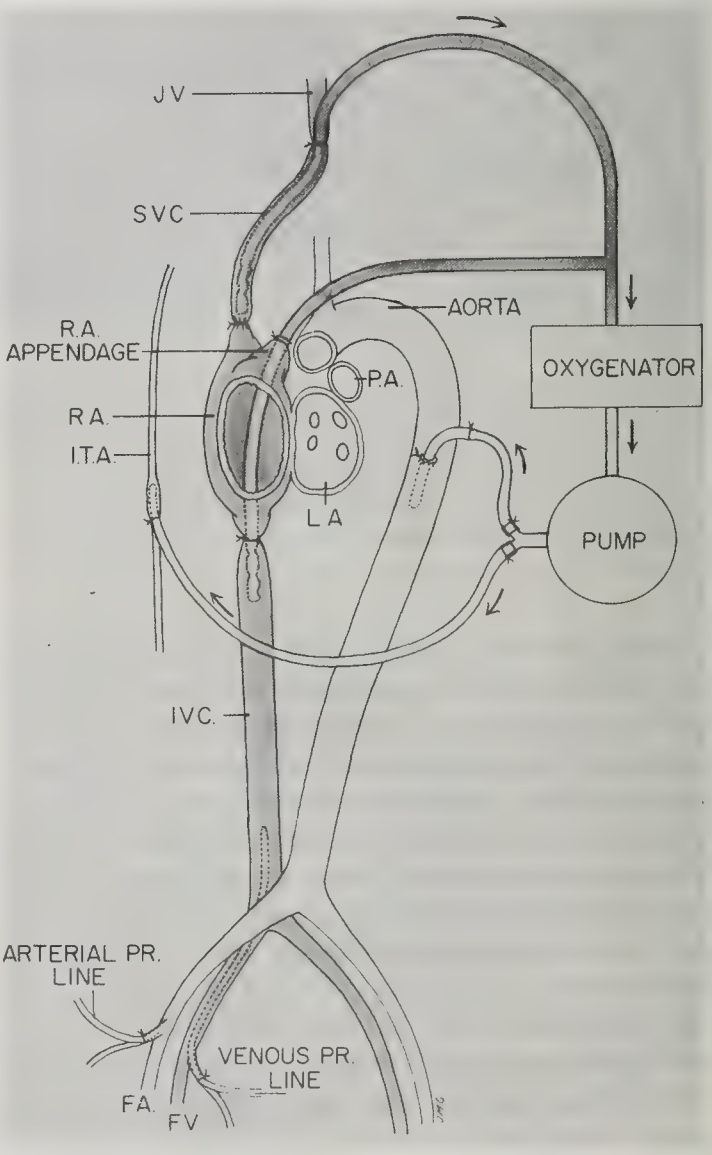


FIGURE 7.—Circuit of extracorporeal circulation during implantation of TAH.

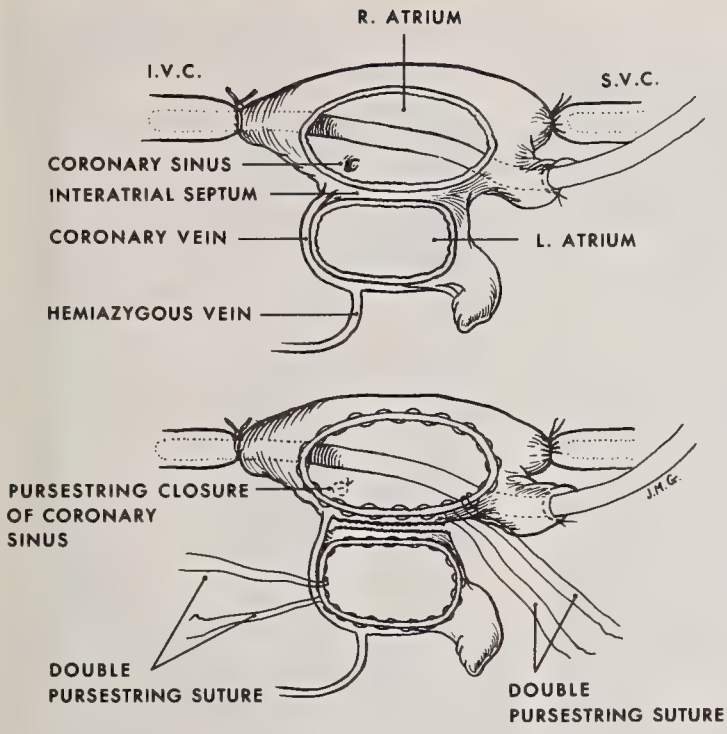


FIGURE 8.—Showing anatomy and surgical procedure in calf after the natural heart has been completely removed. Hemiazygos vein directly drains in the coronary sinus. Lower drawing shows that the interatrial septum has been split providing a sufficient edge for connection of both artificial atria.

chambers and the two artificial vessels has a side-tube at its highest point when the animal is on its back and the chest is open. These tubes were later used for pressure recording. Removal of air was begun from the right side. After the right atrium had been filled with saline through its side tube, the side tube was clamped and the vena cavae released. Air was sucked out of the ventricle with a syringe connected to its side-tube, thereby permitting saline and blood to flow into the ventricle and fill it. The pulmonary artery was unclamped, and the air was sucked from it. Then the ventricle was checked again to make sure that no air was inside.

On the left side, the procedure was almost the same. The left atrium was first filled with blood coming from the lung, and then the left ventricle and the aorta were filled. Later these side tubes were used for pressure monitoring as well as for blood sampling. Immediately after the extracorporeal circulation was stopped, the prosthetic heart was activated. During the first 20 beats, only the descending aorta was opened, and the brachiocephalic artery was kept closed to prevent the brain from getting air which might have been left in the left ventricle. After checking for bleeding or leaks, the apex of both ventricles were fixed to the sternum in order to hold the device in place. Two chest tubes were inserted into the left chest cavity on the ventral side (for blood) and on the dorsal side (for air). Air tubes, side-tubes, and the lead of a flow-meter probe which was placed around the aorta or the pulmonary artery were lead out through the fourth intercostal space. As soon as the chest was closed in routine fashion the animal was placed in a cage in a normal position. The cannula inserted initially in the left femoral vein for monitoring of venous pressure and the cannula in a branch of the left femoral artery for arterial pressure were transferred each to the right atrial line and to the internal thoracic artery which was used for perfusion of the upper part of the body during extracorporeal circulation. Since the level of the anesthesia was maintained as light as possible, animals awoke quickly.

AFTERCARE

When we were using sheep, they were suspended by two wide canvas bands which were placed beneath the chest and the forelegs, and the abdomen and the hind legs. Both edges were hooked to several springs attached to a sturdy frame so that the sheep's body was always maintained about 15 cm above the floor and the feet were touching the floor (Figure 9). A stanchion consisting of two metal bars was fixed around the neck. Thus, the sheep was able to sit and stand, or turn its head, but could not go forward or backward. When the sheep was awake and strong enough to hold its body weight on its legs, the system worked perfectly. However, when the sheep was weak and its whole body weight was supported by the canvas bands, the chest was compressed interfering with respiration. After we changed from the sheep to the calf, hanging of the canvas bands with springs was abandoned. The bands were affixed when the calf was placed in a cage from the operative



FIGURE 9.—Postoperative sheep whose neck is fixed between two metal bars and body is being supported with wide canvas bands. The bands are hooked to several springs which are attached to a sturdy frame.

table, but were used only to help the calf stand when it was not strong enough to do so alone. Presently the calf's movement is controlled by using only a simple stanchion. The left side of the cage has a wall defect through which all tubings and wires connecting the calf to the machine are led so that the calf can stand up or lie down without causing kinking or twisting of the wires and tubings. After the calf has recovered well enough to stand, pressure transducers are fixed to a specially designed harness at the level of the implanted artificial heart so that the calf can stand or lie down without interfering with the pressure recordings.

ANESTHESIA

For implantation of total artificial hearts in a large experimental animal, the authors selected first calves which weighed around 50 to 60 kg. As Larson et al.[1,2] suggested, anesthesia was induced with ether and maintained with Fluothane. McCrady et al.[3] induced anesthesia with a mixture of nitrous oxide and Fluothane and maintained it with the same gases. In each of these reports, the depressive action of barbiturates was stressed. However, Joel and Sautter[4] used pentobarbital sodium in 15 calves with success, but the calves' chests were not opened and the surgical procedure was not extensive.

In our experiments, when ether was used in a calf weighing more than 60 kg, anesthesia deep enough for intubation took 15 to 20 minutes to achieve. With 7 to 8 mg/kg body weight of Nembutal, intubation was possible without difficulty and no laryngeal reflex was noted. Fluothane is an important early cause of difficulty

since deep anesthesia with Fluothane causes extreme decrease of arterial pressure particularly in a very young calf. The arterial pressure itself is the most sensitive index to the depth of anesthesia with Fluothane. Usually a 0.5 per cent concentration of Fluothane was sufficient for anesthesia. Occasionally, especially during total bypass, the flow of Fluothane was completely shut off, to be restarted only at the sign of wriggling or spontaneous movement of the diaphragm.

DISCUSSION

In the first ten calf experiments, the total artificial heart was started immediately after the extracorporeal circulation was stopped. It was found that blood coming back to the left atrium was not oxygenated at all,[5] and none of the calves survived longer than five hours. In order to elucidate this unexpected impairment in pulmonary function, we carried out some experiments in which only the right side of the natural heart was replaced by a pump.[6] We have found that the flow rate at the beginning of pumping of the artificial heart is a critical factor. The pulmonary insufficiency seemed to be due to opening of arterio-venous shunts, because of the considerable increase in pulmonary resistance after a period of complete cessation of pulmonary blood flow during cross clamping of the pulmonary artery while inserting the prosthetic heart. Oxygenation was improved by gradually increasing the cardiac output of the prosthetic heart while the major part of the animal's circulation was maintained by the extracorporeal circulation which was then gradually reduced. The time of safe transition from the extracorporeal circulation to the artificial heart varied from 15 minutes to two hours. Use of vasodilator such as isoproterenol was not effective.

Cumbersome procedures in calves made us change the experimental animal from the calf to the sheep. The sheep has certain advantages over the calf as described by Borrie et al.[8] (1) It is cheap. (2) It is easy to transport. (3) It is easy to maintain in long-term survival experiments. (4) It does not grow to unwieldy sizes. (5) Physiologically its size approximates man. However, after four years of experience with sheep, we found the following drawbacks and went back to the calf. (1) The salivary and respiratory tract secretions were troublesome. (2) Its red blood cells are fragile.[9] (3) Its oxygenation after extracorporeal circulation is poor. (4) It has weak resistance to traumatic shock. (5) Its chest cavity is small for its body weight. (6) It is difficult to obtain large sheep.

Using calves weighing from 70 to 90 kg, we now have no problem with oxygenation immediately after the cessation of extracorporeal circulation. Recently, we have used Surital alone throughout experiments with assist devices without any trouble in postoperative respiration. However, with this agent, we have noted insufficient oxygenation in some total artificial heart experiments. It is obvious that about one dozen tubings and wires traversing the left chest cavity and penetrating the chest wall definitely affects the respiratory function. The left lung is compressed, and the thorax movement is disturbed physically as well as by pain. Although these effects alone would not kill the animal, they are additive to other adverse factors in implantation surgery.

SUMMARY

Sheep and calves to be used for implantation surgery of various types of artificial hearts have been compared from various standpoints.

Anesthesia with a short-acting barbiturate for induction and with Fluothane for maintenance seems satisfactory in large experimental animals.

Surgical implantation techniques of B-LHAD, S-LHAD, and TAH have been discussed in detail.

Control of the movement of postoperative animals for safe monitoring of necessary parameters has been described.

REFERENCES

1. Larson, R. E., Moffitt, E. A., and McGoon, D. C. Experimental cardiac surgery in calves. I. Anesthesia. J. Surg. Res. 3:101, 1963.
2. Larson, R. E., and McGoon, D. C. Experimental cardiac surgery in calves. II. Management for aortic valve surgery. J. Surg. Res. 3:104, 1963.

3. McCardy, J. D., Greenberg, S. D., Hallman, G. L., McNamara, D. G., Rosenberg, H. S., O'Neal, R. M., and Keats, A. S. The use of the newborn calf in cardiovascular research. Cardiovas. Res. Center Bull. Baylor Univ. Coll. Med. 1:110, 1963.
4. Joel, D. D., and Sautter, J. H. Preparation of a chronic thoracic duct-venous shunt in calves. Proc. Soc. Exp. Biol. Med. 112:856, 1963.
5. Akutsu, T., Mirkovitch, V., Topaz, S. R., and Kolff, W. J. Silastic sac type of artificial heart and its use in calves. Trans. Amer. Soc. Artif. Intern. Organs 9:281, 1963.
6. Akutsu, T., Panayotopoulous, E. K., Topaz, S. R., and Kolff, W. J. A pneumatically driven intrathoracic artificial heart. Ann. N. Y. Acad. Sci. 120:766, 1964.
7. Akutsu, T., Mirkovitch, V., Topaz, S. R., and SenGupta, A. Insertion of artificial hearts inside the chest of calves. Ann. Surg. 161:365, 1965.
8. Borrie, J., Lichter, I., and Miller, W. N. experimental heart surgery in sheep. J. Surg. Res. 7:560, 1967.
9. Takano, H., Takagi, H., Turner, M. D., Henson, E. C., Crowell, J. W., and Akutsu, T. Problems in total artificial heart. Trans. Amer. Soc. Artif. Intern. Organs. 17:449, 1971.

DISCUSSION

Chairman Michael P. Kaye: This very fine presentation is now open for discussion. What type of pulmonary pathology did you find in the sheep lungs at autopsy?

Dr. Akutsu: Severe atelectasis and hemorrhage. We divided the sheep into two groups. In the first group we didn't use any particular medication, and we found severe pathological ordination. After that we started using dibenzyline and thorazine, and we noticed some improvement. But we had much less of a problem in this area when we used calves.

Craig Hassler, Battelle Columbus Laboratory: What were the interior surfaces of these hearts and what did they look like after the eight to ten days survival?

Dr. Akutsu: As you know, it is very difficult to keep the surface of silk and rubber clean, and until the device is ready to use for implantation, it has to go through many procedures with prospects for contamination. We coat the entire internal surface just before implantation, so that it's just a smooth surface of silk and rubber. None of the calves died by thrombolysis. After 10 days survival, one of the animals had developed a thrombus formation at the bottom of the aortic valve leaflet. The others stayed very clean, but we found some infarcts in the kidneys.

Victor W. Bolie, University of New Mexico: Would you comment on the types of valves you used and also on the status of the biolyzed material, the aldehyde-treated connective tissue lining for devices?

Dr. Akutsu: Our device uses the oval shape valve opening to save space. In our elliptical valve is a major axis of about 2 inches with one membrane hanging in the ventricle. During contractions, the blood pushes the large membrane against one side of the ventricular wall and the other side of the ventricular wall is pushed against the membrane. It works like a leaflet or bicuspid valve. We call this a bicuspid S-shaped valve, leaflet type, because the closure line looks like an "S." I don't know about the surface of the material. I have experience only with a smooth silk and rubber surface.

Gerald Moss, Troy, New York: Why do you have six chambers in your peristaltic pump? It seems to me you theoretically lose any benefit beyond three.

Dr. Akutsu: Each chamber is approximately 25 cc. After the complete bypass, when the animal or patient doesn't need any more help from the device, we have to wean him from it. But I don't think it is good to put the whole responsibility back to the natural heart suddenly. So to be able to regulate the weaning from the device, we have six chambers so that we can reduce the use of the device one chamber at a time. We also can regulate the weaning from the device by triggering from one to two chambers all the way up to one to five chambers.

Dr. Moss: My point is that if you think in terms of peristalsis, your stroke volume is one chamber and you don't need those distal to it to continue moving the blood along. So your rate can change but your stroke volume is that of one chamber collapsing, and the other three chambers are just redundant.

Dr. Akutsu: I don't know how you can control the cardiac output in one chamber. We consider this peristalsis assist device as a temporary aid, so that we don't have to stick to the

available space, shape, and volume. This pump is completely made in one piece with no connections, and actually the inside of the entire tube is exactly the same cross-sectional area. If you have to design one chamber, it is impossible to make that kind of design.

MIROSLAV KLAIN, Cleveland Clinic: May I ask you what size calf you use for your experiments? In our experience (and I would like to re-emphasize what Dr. Norman said), we use calves weighing about 140 pounds. There are many more complications with calves this size than with calves of about 200 pounds that are about 10–12 weeks of age. I think this is very important point in order to obtain good results.

I would like to refer to one question about the biolyzed heart. I have used this at the Cleveland Clinic and so far have had no thrombolytic complications with it. The inner surface is some specially treated pericardium and the outflow and inflow valves are matured valves from another calf.

DR. AKUTSU: When I was working at the Cleveland Clinic in Dr. Cole's laboratory, we tried using calves weighing 120–130 pounds. With the first 10 calves, we stopped our pump oxygenator suddenly and started the artificial heart at full swing from the beginning. Blood coming back to the left atrium was not oxygenated at all, but was just regular venous blood. We felt that this problem was a result of working with such young calves, and now that we are using calves of 160–190 pounds, we don't have the problem anymore.

EXPERIMENTAL CARDIAC SURGERY IN THE CALF

William J. Donawick and James W. Buchanan*

ABSTRACT

The calf has many advantages as an experimental animal, particularly for intrathoracic and cardiac transplantation studies.

Halothane and nitrous oxide were excellent anesthetics for the induction and maintenance of anesthesia. In contrast, barbiturates caused prolonged depression and high mortality. Particular attention was required to insure adequate ventilation during thoracotomy. Large tidal volume-high pressure ventilation was necessary to provide adequate oxygenation. Pulmonary function was impaired by the accumulation of gas in the rumen and of mucus in airways.

The cellular immune response to heart grafts was prompt and vigorous but could be successfully suppressed with antilymphocyte serum. The administration of immunosuppressants to the calves increased susceptibility to infection and in the case of prednisolone, delayed wound healing.

INTRODUCTION**

The calf is particularly well suited for use in cardiac transplantation trials, for intrathoracic artificial organ studies, and for evaluation of intracardiac prostheses. The heart and major vessels of the calf approximate those of man, are strong, and withstand suturing well. With careful attention to surgical technique, anastomoses remain free of thrombosis. The calf closely resembles man hemodynamically.[1] Blood for transfusion or for priming an extracorporeal oxygenator is readily available from adult cows and, since preformed antibodies to foreign blood antigens do not exist, the blood need not be crossmatched. The calf does not develop dirofilariasis, a common endoparasite that complicates vascular and cardiac surgery in the dog. The calf is docile, reasonably inexpensive, and requires little care.

Unfortunately, investigators have been hampered because not enough was known until recently about inducing and maintaining anesthesia in the calf, about ventilation during thoracotomy, and about techniques for successful cardiopulmonary bypass.[2-4] With the solution of many of these problems, transplantation studies with genetically foreign organs were possible. Long term organ function required the use of immunosuppressants and little was known about the immune response of the calf or the relative merits of the available immunosuppressants.

This paper reviews techniques for inhalation anesthesia, ventilation, and thoracotomy, describes methods for maintenance and treatment of calves, and discusses the effectiveness and limitations of immunosuppressants used in the calf.

MATERIALS AND METHODS

One hundred and eighty calves, 60 to 120 kg, underwent anesthesia and thoracotomy for transplantation of prosthetic and allograft heart valves and heterotopic xenograft hearts. Anesthesia was induced and maintained with nitrous oxide and halothane[5] through either a Chicago or a North American Drager[A] anesthetic apparatus with a Fluotec[B] or Vapor[A] halothane vaporizer, and a Bird[C] Mark IV assistor-controller powered by a Bird Mark VII or IX pressure-sensitive respirator. After induction, the calves were intubated with a 10–13 mm i.d. cuffed endotracheal tube 40 cm in length.

* School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania 19348

** Supported by U.S. Public Health Service Grant 5-RO1-HE-12850 from the National Heart Institute, by a Grant-in-Aid from the Delaware Heart Association, and by private donations to the Heart Surgery Fund of New Bolton Center. Dr. Buchanan is recipient of a Career Development Award, National Heart Institute, USPHS, Grant No. K4-HE-13903.

[A] Narkovet, North American Drager, P. O. Box 121, Telford, Pa. 18969.

[B] Fluotec Vaporizer, Cyprane Limited, Haworth, Keighley, England.

[C] Bird Corporation, Palm Springs, California.

Horse anti-bovine lymphocyte serum (ALS) was prepared by the injection of bovine lymphocytes in horses. The lymphocytes were collected, by the technique of Chanana and Cronkite,[6] from the thoracic duct of clinically normal calves. The lymphocytes were separated from the lymph by low speed centrifugation, washed twice in Hank's balanced salt solution and injected into horses. On 3 occasions, 14 days apart, each horse received approximately 75×10^9 viable lymphocytes by intravenous injection and 7 to 10 days after the third injection it was exsanguinated. The blood was allowed to clot and the serum separated and heated to 56° C for 30 minutes to inactivate complement. Merthiolate 1:10,000 was added as a preservative and the serum was stored at −10°C.

Treatment of calves with ALS was started 6 days before transplantation of allografts and xenografts. The calves were given approximately 2 ml/kg of ALS subcutaneously either daily or every second day.

The calves given prednisolone acetate received 1 mg/kg daily; the time of the first dose ranged from 2 days before to 2 days after surgery. Dosage was reduced to 0.5 mg/kg 14 days after transplantation.

To study the host's immunologic response to a heart xenograft and determine the capability of ALS to suppress rejection, goat hearts were transplanted, heterotopically to the thorax of calves. A technique was developed that permitted transplantation of the goat heart with less than 30 minutes ischemia after it was taken from its primary host. With this technique, the goat heart was removed and flushed with 1 liter of saline at 4° C containing 10 units/ml of heparin. The brachiocephalic and pulmonary arteries were joined by end-to-side anastomoses to the brachiocephalic and pulmonary arteries of the calf through a left intercostal thoracotomy.

RESULTS AND DISCUSSION

Male calves of dairy breeds (Holstein, Ayrshire, or Guernsey) weighing 160 to 180 pounds were preferred to calves of beef breeds. Beef calves were undesirable because they were more difficult to handle, had a smaller thoracic cavity and had more perivascular fat which complicated dissection. Males were preferred as females were often culled from a herd because of illness, whereas male calves were commonly reared carefully for veal production and were sold in excellent condition. Calves in excess of 150 pounds were usually weaned from milk (or a milk substitute) and were functioning as ruminants. Thus, they required only grain,[D] hay, water and salt[E] *ad libitum*. In addition, these older calves seemed more resistant to pulmonary complications during ventilation and cardiopulmonary bypass. We found it better to use weaned calves that occasionally bloated (see below) because they were less prone to pulmonary complications during surgery.

During inhalation anesthesia, ventilation and thoracotomy special attention must be given to: (1) controlling ruminal distension (bloat) and excessive secretion that may obstruct airways, (2) induction of anesthesia, (3) intubation, hay, water and salt[E] *ad libitum*. In addition, and (4) maintenance of normal acid-base balance and oxygen tension.

Abdominal distension in the calf results from continuous fermentation and accumulation of gas in the rumen during surgery.[7] Because of the calf's rather small thoracic cavity, abdominal distension severely impairs pulmonary function and decreases venous return from the caudal vena cava. Ruminal distension and the possibility of regurgitation and inhalation pneumonia during induction of anesthesia were reduced by withholding food for 24 hours before surgery to decrease the amount of fermentable material. In addition, the rumen, which is on the left side, was decompressed if it became distended during or after surgery; this was done with a 14 gauge, 2 inch needle, inserted into the rumen through the left flank caudal to the last rib. A piece of rubber tubing was attached to the needle and the free end was placed under water to control the odor.

During anesthesia there was increased secretion of saliva and tracheal and bronchial mucus which if allowed to accumulate would have impaired ventilation. Secretion was effectively stopped by the preanesthetic administration of

[D] Calf Startena, Ralston Purina Co., Checkerboard Square, St. Louis, Missouri.

[E] Salt Brick Morton Salt Company, Chicago, Illinois.

0.05 to 0.1 mg/kg of atropine sulfate 30 minutes before surgery.

Barbiturates, even the very short-acting ones, are unsafe in the calf because they cause prolonged depression and high mortality.[8] The administration of halothane and nitrous oxide through a face mask offers a safe, rapid alternate method for the induction of anesthesia. For induction, a conical face mask was fashioned from a plastic detergent bottle with a foam rubber seal at the muzzle end and an adaptor for attaching it to the anesthetic machine at the spout. With the calf standing, anesthesia was begun with halothane (4%), nitrous oxide (71%), and oxygen (25%). The calf was placed in lateral recumbency when it was no longer able to stand even with assistance. This prevented the involuntary movements of the excitatory stage. Approximately 5 minutes were required before the laryngeal reflex was abolished sufficiently to permit intubation.

Inability to see the larynx because of the length of the calf's muzzle and the thickness of the base of the tongue made endotracheal intubation difficult. It was most easily accomplished by blind placement of the endotracheal tube with the calf lying on its right side, the head hyperextended and the tongue pulled forward as far as possible. A mental stylet was placed in the endotracheal tube to aid in the manipulations to get past the epiglottis.

Moderate to severe respiratory acidosis occurred during inhalation anesthesia in calves that were breathing spontaneously even though they were kept in the first stage of surgical anesthesia.[9,10] Oxygenation during spontaneous ventilation before thoracotomy was adequate with 40% oxygen in the gas mixture.

Large tidal volume-high pressure (30 to 33 cm of H_2O) ventilation was necessary to provide adequate oxygenation during thoracotomy. The tidal volume required was approximately 2 liters with a minute-ventilation of 40 liters. An undesirable side-effect of hyperventilation was respiratory alkalosis, but it was inevitable if adequate oxygenation were to be assured. The failure to maintain adequate oxygenation during thoracotomy without large tidal volume-high pressure ventilation was probably caused by a combination of factors, including the anatomic structure of the bovine lung and the lateral positioning of the calf during thoracotomy. Mechanical damage was not seen in the lungs of calves ventilated continuously at a high pressure. Small tidal volume-low pressure ventilation during the thoracotomy, even with 100% oxygen, resulted in hypoxemia, hypercapnia, atelectasis, and increased alveolar-arterial oxygen gradient.

The thymus gland occupies a large part of the cranial thorax in young calves. If thymectomy is attempted, care must be taken during dissection because the phrenic and vagus nerves course through it and the gland is supplied by one or more blood vessels easily torn from the brachiocephalic trunk.

The brachiocephalic trunk is the only branch arising from the ascending aortic arch. The calf has a left azygos vein which empties into the coronary sinus; for this reason coronary sinus blood does not give a true reflection of myocardial metabolism.

A median sternotomy is easy to perform and provides access to both sides of the thorax. However, the calf has a deep thorax and accessibility to the heart is poor. Postoperatively, infection of the incision line often occurs since calves spend much of their time in sternal recumbency and contamination of the incision is unavoidable.

A lateral thoracotomy without rib resection in the left 3rd intercostal space provides good exposure of the brachiocephalic trunk and the cranial aspect of the main pulmonary artery. The left 4th space is preferable for approaching the pulmonic valve and cannulating the right atrium. The left 5th space is preferable for approaching the mitral valve. The skin incision was always made over the 6th intercostal space. If placed further forward it often became infected and healed slowly.

Frequently after operation, the calves were anorectic. At the commencement of the operation we administered an electrolyte solution[F] intravenously at the rate of 80 ml/kg/24 hr. The fluids were given until the calf was able to drink sufficient water (approximately 10% of body weight in water/day).

[F] Normosol R and M, Abbott Laboratories, North Chicago, Illinois 60064.

Diarrhea was a particularly serious condition in the calf. It resulted in rapid dehydration and severe metabolic acidosis. When it occurred, fluid was given intravenously at a rate of up to 150 ml/kg/24 hr, depending upon the severity of the diarrhea. The metabolic acidosis was treated with sodium bicarbonate based on either acid-base determinations on arterial blood or on estimates of bicarbonate deficit.[11]

Immediately after operation the calves were placed in sternal recumbency and the lungs were hyperinflated to expand areas of atelectasis. The endotracheal tube was removed, after the laryngeal reflex had returned, without deflating the cuff so as to remove any ingesta that had accumulated in the trachea above the cuff. The calf was given oxygen through a nasal tube if after surgery the PaO_2 of the calf while breathing room air was less than 70 mm. Hg. Five liters per minute of oxygen were administered through a polyethylene catheter placed approximately 15 cm. into a naris and secured to the external naris with a simple interrupted suture.

During intrauterine development, the calf produces only small amounts of immunoglobulins[2-14] insufficient to protect it against postnatal infection. Thus, for defense against diseases, the neonatal calf must rely on the immunoglobulins obtained soon after birth from the dam's colostrum.[15,16] When a related ruminant, the fetal lamb, is given allogeneic skin grafts at 85 days or later in gestation they are rejected and have a mononuclear cell infiltrate.[17] The cellular immune response of the neonatal calf to genetically foreign skin and organ grafts is prompt and vigorous and is presumably present *in utero*. A breed difference has been noted in the humoral antibody response of newborn calves.[18]

Suppression of the cellular immune response of calves with a number of immunosuppressive agents has been reported. It has been possible to prolong the survival of allografts and xenografts and tumor transplants with whole-body irradiation,[19] by depletion of lymphocytes,[20] and with antilymphocyte serum (ALS) either alone or with prednisolone.[21]

Marshak et al.[19] successfully transplanted lymphosarcoma from cows to unrelated calves given 200 rads of whole-body irradiation but the rate of success was low, and the mortality rate from the effects of irradiation was high.

Depletion of lymphocytes by extracorporeal irradiation of lymph[20] prolonged the survival of skin allografts for 20 days. This technique, however, is impractical for organ transplant studies.

In the calf ALS is superior to other immunosuppressants.[21] It has prolonged the survival of skin allografts and xenografts and heart xenografts. Its use also permitted the successful transplantation of bovine lymphosarcoma.[22]

The ALS caused a febrile response, depression of the total leukocyte and absolute lymphocyte counts (Figure 1). Discontinuation of ALS treatment resulted in graft rejection after a gradual increase in the total leukocyte and absolute lymphocyte counts. Continued administration of ALS resulted in thrombocytopenia and a gradual decrease in the packed cell volume. During ALS treatment calves were able to respond to bacterial infection with a neutrophilia (Figure 2).

Bacterial infections in ALS-treated calves were, in most instances, easily controlled with antibiotics. It was impossible to prevent or treat, however, polyarthritis caused by *Mycoplasma bovimastitidis* in ALS-treated calves. The onset of the arthritis was sudden, and

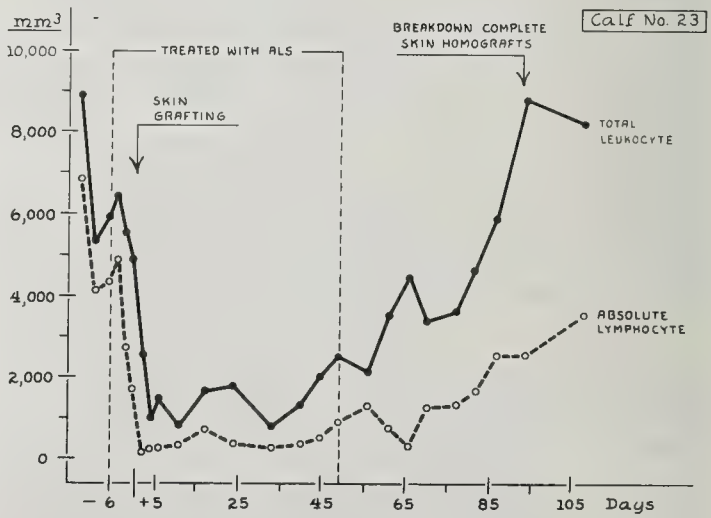


FIGURE 1.—Changes in the total leukocyte and absolute lymphocyte counts of calves given 2 ml/kg of ALS every second day.

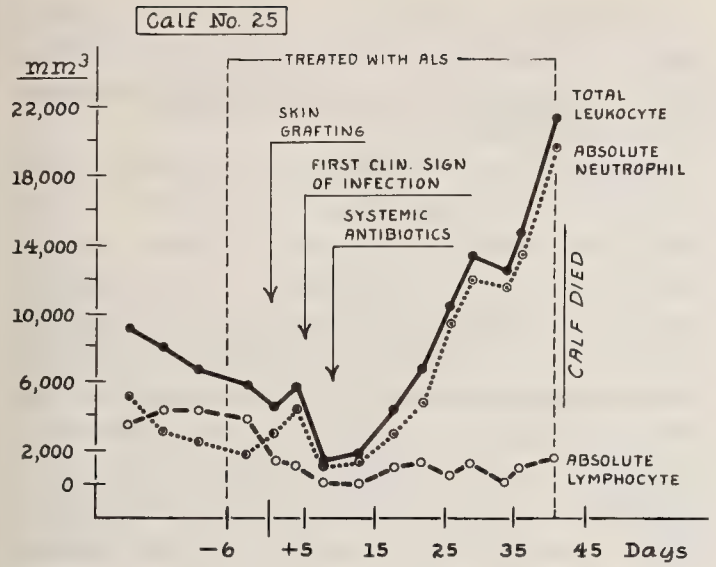


FIGURE 2.—A calf that developed multiple subcutaneous abscesses caused by *Corynebacterium pyogenes* during treatment with ALS. Note the elevated total leukocyte and absolute neutrophil counts with continued suppression of absolute lymphocyte counts.

within a matter of days, the calves were totally and permanently incapacitated.

The use of prednisolone as the sole means of suppressing rejection of goat hearts in calves did not appreciably prolong survival but when combined with ALS it prevented rejection.[21] However, the surgical wounds did not heal, and we were unable to control bacterial and fungal infections.

REFERENCES

1. WEBER, K. T., DENNISON, B. H., FUQUA, J. M., SPEAKER, D. M., and HASTINGS, F. W. Hemodynamic measurements in unanesthetized calves. J. Surg. Res. 11:383, 1971.
2. LARSON, R. E., MOFFITT, E. A., and MCGOON, D. C. Experimental cardiac surgery in calves. I. Anesthesia. J. Surg. Res. 3:101, 1963.
3. LARSON, R. E., and MCGOON, D. C. Experimental cardiac surgery in calves. II. Management for aortic valve surgery. J. Surg. Res. 3:104, 1963.
4. NELSON, R. J., MOHRI, H., DILLARD, D. H., and MERENDINO, K. A. Heterotransplantation of the human pulmonic valve to the calf pulmonic area. J. Thorac. Cardiovasc. Surg. 52:863, 1966.
5. SHORT, C. E. Bovine inhalation anesthesia. J.A.V.M.A. 157:1571, 1970.
6. CHANANA, A. D., and CRONKITE, E. P. Semipermanent extracorporeal arteriovenous and thoracic duct—venous shunts in cattle. Am. J. Vet. Res. 27:683, 1966.
7. DONAWICK, W. J., NARA, Y., and BAUE, A. E. Technique for successful cardiopulmonary bypass in the calf. J. Surg. Res. 9:79, 1969.
8. JENNINGS, S. The use of B. B. methyl ethyl glutarimide for terminating barbiturate anesthesia in calves. Vet. Rec. 70:494, 1958.
9. DONAWICK, W. J., and BAUE, A. E. Blood gases, acid-base balance and alveolar arterial oxygen gradient in calves. Amer. J. Vet. Res. 29:561, 1968.
10. DONAWICK, W. J., HIREMATH, I., and BAUE, A. E. Anesthesia, ventilation and experimental thoracotomy in the calf. Amer. J. Vet. Res. 30:533, 1969.
11. DONAWICK, W. J., and CHRISTIE, B. A. Clinicopathologic conference—Neonatal diarrhea in the calf. J.A.V.M.A. 158:501, 1971.
12. SCHULTZ, R. D., DUNNE, H. W., and HEIST, C. E. Ontogeny of the bovine immune response. J. Dairy Science 54:1314, 1971.
13. MERRIMAN, J. G. S. Serum immunoglobulins in newborn calves before, and after colostrum feeding. Can. J. Comp. Med. 35:269, 1971.
14. CLASSICK, L. G., and FERNELIUS, A. L. Bovine viral diarrhea virus: Neutralizing antibodies in a calf obtained by cesarean section from cow which was infected. Am. J. Vet. Res. 31:393, 1970.
15. KRUSE, V. Absorption of immunoglobulin from colostrum in newborn calves. Animal Prod. 12:627, 1970.
16. SELMAN, I. E., MCEWAN, A. D., and FISHER, E. W. Absorption of immune lactoglobulin by newborn dairy calves. Res. Vet. Science 12:205, 1971.
17. PRENDERGAST, R. A., SILVERSTEIN, A. M., and PARSHALL, C. J. Immunoglobulin production and allograft rejection in the fetal lamb. Transplantation 8:540, 1969.
18. SCHULTZ, R. D., DUNNE, H. W., and HEIST, C. E. Breed difference in the immune response of newborn calves. Am. J. Vet. Res. 32:1337, 1971.
19. MARSHAK, R. R., HARE, W. C. D., DODD, D. C., MCFEELY, R. A., MARTIN, J. E., and DUTCHER, R. M. Transplantation of lymphosarcoma in calves. Cancer Res. 27:498, 1967.
20. CHANANA, A. D., BRECHER, G., and CRONKITE, E. P. Histologic observations on skin autografts, isografts, and allografts in cattle. Am. J. Path. 49:1125, 1966.
21. DONAWICK, W. J., SHAFFER, C. F., DODD, D. C., BUCHANAN, J. W., and FREGIN, G. F. Cardiac and skin heterograft rejection: Suppression with antilymphocyte serum: Proc. Transpl. Soc. 3:551, 1971.
22. DONAWICK, W. J., JOHNSTONE, C., MARTENS, J. G., DODD, D. C., MARTIN, J. E., and MARSHAK, R. R. Successful transplantation of lymphosarcoma in calves treated with antilymphocyte serum. J. Nat. Cancer Inst. 44:467, 1970.

DISCUSSION

CHAIRMAN MICHAEL P. KAYE: Thank you very much Dr. Donawick. The paper is open for discussion. May I ask that discussants identify themselves by name and home city.

STAN ALLEN, Salt Lake City: What dose of heparin do you use for your calves just before they go on extracorporeal circulation?

DR. DONAWICK: We haven't done extracorporeal circulation procedures in a number of years, but we used 3–5 milligrams per kilogram when we did. At that time we had a problem with platelet aggregations on the oxygenators, and we attempted to remedy it by using increased doses of heparin. I don't know whether or not that is the current thinking, and would refer questions on heparin use and dosage to Dr. Ross.

C. GRANT LA FARGE, Children's Hospital Medical Center, Boston, Mass.: I have a comment for those of you who are still using the barbiturates, notably sodium thiopental or pentobarbital. We've done studies with about 500 calves, and our routine regimen is to begin anesthesia with sodium thiopental and maintain it with halothane.

KARL T. WEBER, Birmingham: I'd like first to laud Dr. Donawick for having the courage to begin studying these physiological questions some years ago. When we first began our work 2½ years ago, we were in constant consultation with him. We have now used some 200 calves, and I'd like to comment on our experiences with anesthesia. With the use of intravenous ketamine in doses of 2 milligrams per kilogram for both the induction and maintenance of anesthesia, we've been able to have the animals standing within 30 minutes after open thoracotomy with a great deal of instrumentation in the heart. We've also been able to discard the volatile anesthetics and their attendant equipment, and have eliminated the need for an anesthetist.

I'd also like to echo Dr. LaFarge's comments about the lethality of the barbiturate anesthetics and their marked suppression of post-operative recovery in calves.

GERALD MOSS, Rensselaer Polytechnic Institute and Albany Medical College: We've used about 400 calves in the last 13 years, and I'd like to make a few comments on our experiences. The veterinarians and the veterans of bovine surgery have a barbiturate cocktail which seems to work, although it may be that the other anesthetics are better. In this commercially available mixture, the barbiturate is combined with magnesium sulfate and chloral-hydrate, and the necessary amount of barbiturate is thereby reduced.

I'd also like to mention the Jersey cow, which is about the smallest of the dairy breeds and has a newborn calf that weighs 25–40 pounds. An animal this size is comparable to the dogs you have used, and is sometimes more convenient to work with than a larger animal, and fresh blood is readily available at blood banks. The male calves of a dairy breed are cheaper than dogs in our area because they are not used for breeding and don't give much meat.

We too have found that the heparin requirements have gone up. We empirically use about quadruple the amount on a weight basis that we'd use for humans or dogs. We work with day-old or month-old animals, and we have noted that the younger the animal, the greater our problem with lung changes. We have found the lung lesion to be related to brain oxygenation. We can induce this lung lesion, which looks just like hyaline membrane disease, in 15 minutes with cerebral hypoxemia. Although it would appear that high inspiratory pressure might very well be a way of avoiding this lesion, the mechanism seems to be centrally neurogenic. And we do get hemorrhage and atelectasis, as well as edema and hyaline membranes within 15–20 minutes in the newborn with cerebral hypoxemia.

REGIONAL DENERVATION OF THE CANINE HEART

Geis, W. P. and Kaye, M. P.

If implantation of cardiac and paracardiac devices interferes with autonomic cardiac innervation, neural control of cardiovascular events is altered. Accordingly, the sites of entry onto the heart of cardiac efferent nerves must be well defined. Cardiac inotropic and chronotropic innervation was evaluated using electrical stimulation of efferent cardiac nerves. Stimulations were repeated following localized transection of paracardiac tissue. Results indicate that efferent nerves enter the heart along the superior vena cava, subjacent to the adventia of the pulmonary artery and aorta, along the superior and lateral margins of the left atrium, and at the junction of the inferior vena cava with the left atrium. These areas must be assiduously avoided during paracardiac and cardiac manipulation and/or instrumentation during experimental investigations.

INTRODUCTION

In recent years, investigators studying cardiovascular hemodynamics, ventricular mechanics, atrioventricular conduction and myocardial excitability, as well as cardiovascular reflex phenomena have made concerted efforts to assidously avoid the alterations in cardiovascular physiology which occur during anesthesia. Anesthetic agents have been well-known to suppress autonomic responses[1] and to depress myocardial contractility directly.[2] To this end, a variety of invasive techniques have been utilized to implant blood flow devices, pressure gauges, venous and arterial occlusion cuffs, and various infusion and sampling instruments. The experimental animals—chronically instrumented—are later subjected to experimental procedures, and investigations are carried out with animals in the awake state. These investigational data are not only unmodified by anesthetic agents but physiological alterations induced by the open-chest state and positive pressure respiration are avoided.

The criticism of chronic cardiac and paracardiac instrumentation is subtle but very real. Since reflex phenomena determine the modulation of cardiovascular events during stress and during investigational interventions, it is of paramount importance that implantation of paracardiac devices does not interfere with neural control of the heart and vascular systems. It is, therefore, appropriate in the research setting to be acutely aware of the paths which cardiac autonomic efferent nerves travel to enter the heart. Overzealous dissection in areas which have high concentration of nerve fibers will certainly result in inadvertant denervation of myocardium and/or nodal tissue. Detailed anatomical descriptions of paracardiac and cardiac autonomic nerve pathways have been published.[3] However, until recently, there has been no correlation from the anatomical to the physiological. Randall has provided a physiological demonstration of the segmental nature of myocardial innervation,[4] implying that a portion of the myocardium might be denervated while the innervation to the remainder of the ventricular chambers remains intact. Kaye et al. have interrupted efferent sympathetic nerves which enter the heart subjacent to the adventitia of the great vessels and have obtained functional evidence for sympathetic denervation of the anterior and lateral walls of the ventricular chambers as well as significant depression of myocardial catecholamine content in the corresponding areas.[5] Their co-workers have provided evidence for depression of left ventricular function following acute partial denervation of the left ventricular chamber.[6]

The total hemodynamic impact of inadvertant or purposeful interruption of a portion of the cardiac nerve pathways as they enter or exit the heart is not known. Accordingly, it is the purpose of this presentation: (1) to elucidate the sites of entry of efferent nerves onto the heart along with their corresponding distribu-

*Illinois Institute of Technology Research Institute Chicago, Illinois and Loyola University Medical Center, Maywood, Illinois

tion to myocardium and nodal tissue, and (2) to provide models for preparation of animals with well-defined partial cardiac denervation.

METHODS

Seventeen mongrel dogs were pre-medicated with phencyclidene hydrochloride (2 mg/kg I.M.) and anesthetized with α-chloralose (80 mg/kg I.V.). Positive pressure respiration was instituted, the chest was opened bilaterally, and a pericardial cradle constructed. Walton-Brodie strain gauge arches were sutured to the right atrium (RA), left atrium (LA) and right ventricle (RV). Systemic pressure was monitored from a cannula in the femoral artery. All data were recorded on a Model 1 Grass Polygraph.

Both vagosympathetic trunks were transected in the neck, the anterior and posterior ansae were divided bilaterally at their origin from the stellate ganglia and the mediastinal cardiac nerves were isolated within the mediastinum for subsequent stimulation. The nerves were designated by the terminology of Mizeres:[3] anterior and posterior ansae, recurrent cardiac, craniovagal, caudovagal, stellate cardiac, ventrolateral cardiac (VLCN), ventro-medial cardiac (VMCN), innominate, cervical vagosympathetic trunks and the thoracic vagus nerves.

A series of ablation procedures was performed to selectively interrupt extrinsic cardiac nerve pathways as they enter the heart. Each of the autonomic nerves with cardiac efferent distribution were electrically stimulated supramaximally with square wave impulses of 10 Hz, 5 msec duration and 6 volts prior to intervention. Each of the nerves was subsequently stimulated following each of the ablation procedures.

Figure 1 depicts the interventions: (1) transection and re-anastomosis of the intra-pericardial superior vena cava (SVC) from its junction with the superior margin of the left atrium to the anterior SVC; (2) transection and re-anastomosis of the lateral portion of the intra-pericardial SVC from the anterior SVC to the level of the right superior pulmonary vein; (3) excision of neural elements, epicardium and areolar tissue in the inter-atrial groove from the right superior pulmonary vein to the right inferior pulmonary vein (inter-atrial groove [IAG]); (3) transection and re-anastomosis of the anterior border of the LA from the margin of the inferior vena cava (IVC) with the LA to the left inferior pulmonary vein; (5) transection and re-anastomosis of the lateral border of the LA from the left inferior pulmonary vein to the left superior pulmonary vein; (6) transection and re-anastomosis of the superior border of the LA in the transverse sinus from the left superior pulmonary vein to the medial SVC; (7) circumferential transection of adventita and neural elements on the left half of the ascending pulmonary artery; (8) circumferential transection of adventitia and neural elements on the right half of the pulmonary artery and on the left half of the ascending aorta; (9) transection of the VLCN as it enters the heart at the origin of the left superior pulmonary vein; (10) circumferential transection of adventitia and neural elements on the right half of the ascending aorta. In six experiments the procedures were performed in the order described above; in six further experiments the procedures were performed in the reverse order; in the remaining five experiments the areolar tissue and neural elements located at the junction of the IVC

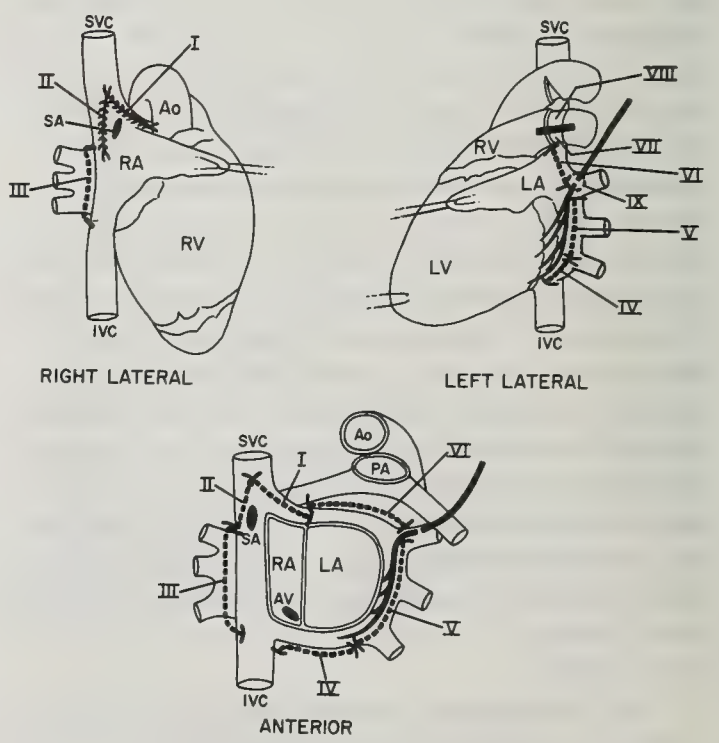


FIGURE 1.—*Regional Denervation of the Canine Heart.* Method of Regional Cardiac Denervation

with the inferior margin of the LA were excised after procedures (1), (2) and (3) to definitively localize neural pathways to this area.

RESULTS

Whenever nerve stimulation resulted in an increment in heart rate accompanied by electrical activity and contraction of the RA and LA preceding the RV electrical activity and contraction, the interpretation was sinus acceleration and sympathetic innervation of the sinus node, whereas, if RA contraction and RA electrical activity occurred at the same time as or slightly following RV electrical activity and contraction, the designation was AV junctional rhythm and sympathetic innervation of the AV node. When nerve stimulation resulted in a decrement in atrial rate, the interpretation was sinus bradycardia or arrest and cholinergic innervation of the sinus node; when stimulations caused no change in atrial rate but slowing of RV rate or complete RV arrest, the response was interpreted as cholinergic innervation of AV node.

Sinus Node Sympathetic Innervation

Prior to ablation procedures, heart rates averaged 139/minute (range 128 to 171). Electrical stimulation of the following nerves resulted in sinus tachycardia, right anterior ansa, right posterior ansa, right stellate cardiac, left posterior ansa, left anterior ansa, recurrent cardiac nerve, innominate nerve, VMCN and VLCN. Following transection of the medial and lateral portions of the SVC, stimulation of the right anterior and posterior ansae, the recurrent cardiac nerve, the innominate nerve, and the right stellate cardiac nerve no longer produced sinus acceleration in all experiments.

Following transection of the SVC, sinus acceleration continued to be observed occasionally during nerve stimulation but the response was of lesser magnitude: left posterior ansa in 2 experiments (HR = 152 and 160), left anterior ansa in 2 experiments (HR = 140 and 152). Following interruption of neural elements at the origin of the great vessels the responses no longer occurred during stimulation of these nerves. Stimulation of the VLCN continued to result in sinus acceleration in *2* experiments (HR = 152 and 193), and the VMCN did so in *1* experiment: (HR = 172). The responses in these experiments were interrupted following interruption of neural elements in the inter-atrial groove.

Figure 2 depicts the response to right stellate cardiac nerve stimulation prior to transection of the SVC (control) and after transection of the SVC (SVC transection). Recordings are right atrial contraction (RAF), electrical activation of the RA (BE [RA]), right ventricular contraction (RVF) and electrical activation of the right ventricle (BE [RV]). Prior to transection of the SVC, the onset of RA electrical and mechanical activity precedes that of the RV. During stimulation there is no augmentation in contractile force, but heart rate (HR) accelerates from 150/minute to 272/minute, and RA activity continues to precede RV activation. This nerve, therefore, contains sympathetic fibers to the sinus node only. Following SVC transection, no longer is HR increased during stimulation. Thus, sympathetic fibers from the right stellate cardiac nerve to the sinus node enter the heart along the anterior aspect of the SVC.

Sinus Node Cholinergic Innervation

Initial electrical stimulation of the following nerves caused sinus depression in all animals: right vagosympathetic trunk, left vagosympathetic trunk, right thoracic vagus, left thoracic vagus and cranio-vagal nerve. Further, recurrent cardiac nerve stimulation resulted in a decrement in sinus rate in 8 experiments while stimulation of the caudovagal nerve did so in 16 experiments. Stimulation of the remaining nerves did not result in a decrement in sinus rate.

Following transection of the SVC, interruption of the sinus slowing response occurred in the maority of nerve stimulations. Decrement in sinus rate did persist on occasion and the response was interrupted as follows: right vagosympathetic trunk required inter-atrial groove dissection on two occasions; left vago-

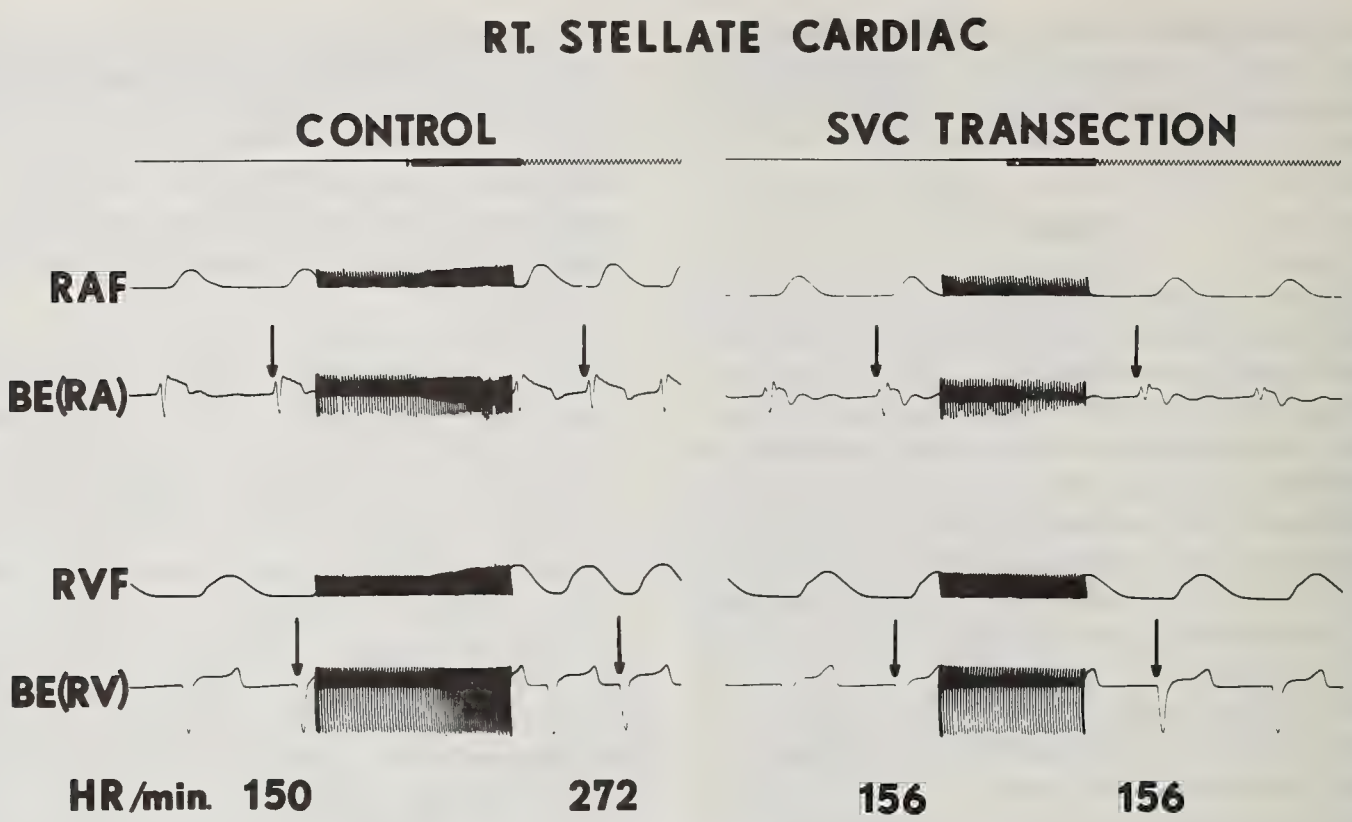


FIGURE 2.—*Regional Denervation of the Canine Heart.*
Rt. Stellate Cardiac Nerve Stimulation
RAF=rt. atrial contractile force
BE(RA)=bipolar electrogram, rt. atrium
RVF=rt. ventricular contractile force
BE(RV)=bipolar electrogram, rt. ventricle

sympathetic trunk required IAG dissection on one occasion and transection of the superior LA on one occasion; right thoracic vagus nerve required IAG dissection on two occasions; left thoracic vagus nerve required IAG dissection on two occasions and transection of the superior LA on two occasions; craniovagal nerve required transection of the superior LA on two occasions; and caudovagal nerve required transection of the superior LA on two occasions. Subsequent to these interventions, on no occasion did electrical stimulation of cardiac nerves result in sinus bradycardia or arrest.

Figure 3 depicts, in control column, a decrease in HR from 141 to 109/minute as well as a depression in RA and LA contractility (RAF and LAF) during electrical stimulation of the left cervical vagus nerve. Following transection of the left SVC (column—left SVC) HR no longer is altered by stimulation; however, depression of contractile force in the RA and LA continues. These results indicate that parasympathetic fibers from this nerve reach the sinus node by entering along the left SVC, and further, the parasympathetic components to the LA and RA do not enter along this route.

Atrial Innervation: Sympathetic

Initial electrical stimulation of the following nerves resulted in augmentation in contractility in both the RA and LA in all experiments: right anterior ansa, right posterior ansa, left anterior ansa, and left posterior ansa. In contrast, stimulation of the VLCN resulted in augmentation of LA contractility in all animals with augmentation in the RA in 7 of 17 experiments. VMCN stimulation produced augmentation in RA in 8 of 17 experiments and augmentation in LA in 3 of 17. Right stellate cardiac nerve stimulation augmented contractility in the RA in 3 experiments and in no experiments was contractility augmented in LA. Stimulation of the

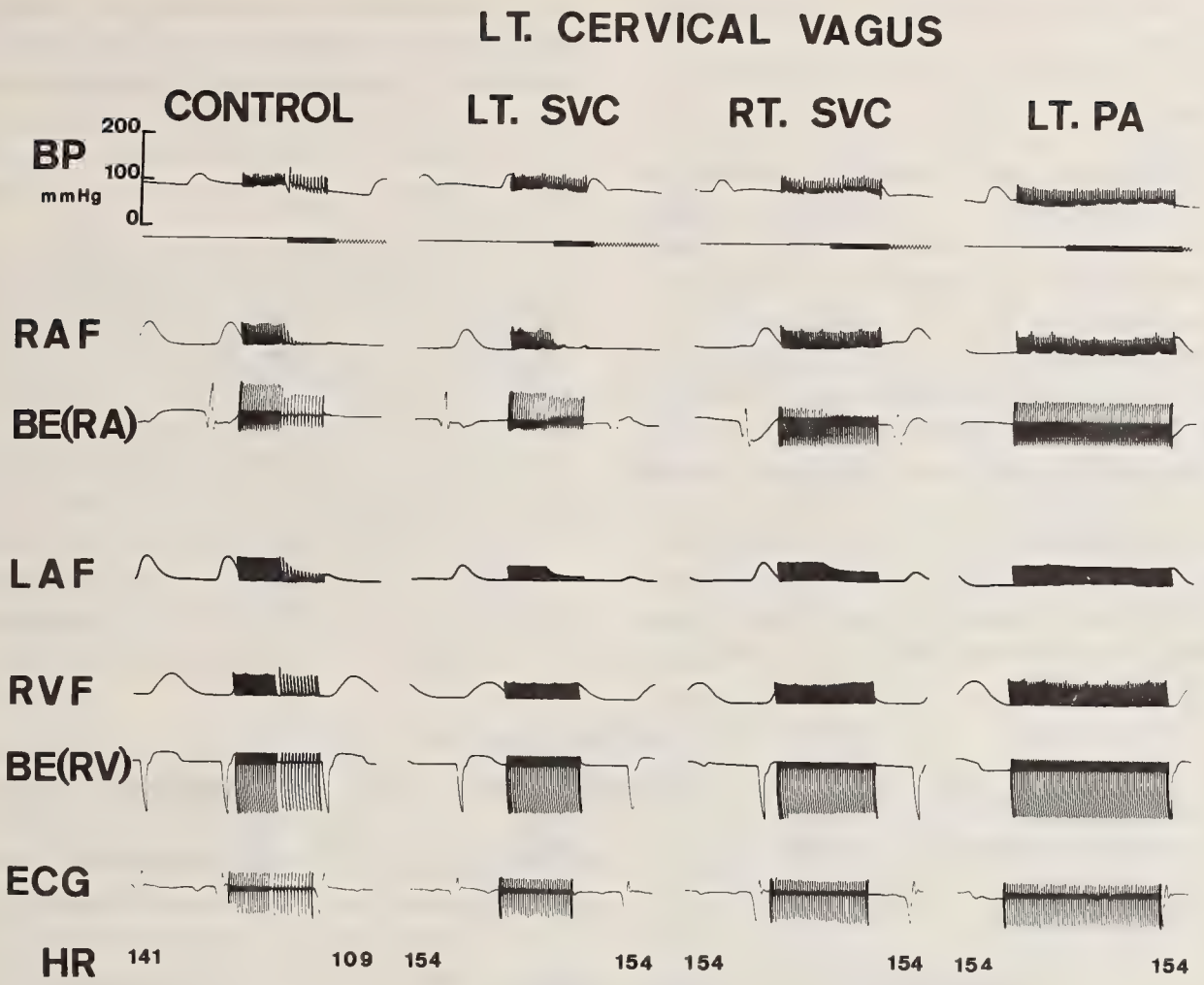


FIGURE 3.—*Regional Denervation of the Canine Heart.*
Lt. cervical vagus stimulation
BP=blood pressure;
RAF=rt. atrial contractile force
BE(RA)=bipolar electrogram, rt. atrium
LAF=lt. atrial contractile force
RVF=rt. ventricular contractile force
BE(RV)=bipolar electrogram, rt. ventricle

remaining nerves did not result in augmentation of contractility in either atria.

Right Atrium

On all occasions, augmentation of RA contractility due to stimulation of the right stellate cardiac nerve was interrupted by transection of the SVC. Innominate nerve or recurrent cardiac nerve augmentation of RA was interrupted on all occasions by transection of the medial SVC or transection of the superior LA. Interruption of augmentation of the RA during stimulation of the VMCN and VLCN was accomplished by either transection of the medial SVC or by interruption of nerves along the root of the great vessels. Only rarely did a single intervention interrupt the positive inotropic response to stimulation of the right anterior ansa, the right posterior ansa, the left anterior ansa and the left posterior ansa. The magnitude of inotropic response to each of these nerves was usually depressed after any one of the following interventions: transection of the SVC, transection of the superior LA, or interruption of nerves along the root of the great vessels. However, following all three of the named procedures, interruption of augmen-

tation of contractility in the RA was invariably accomplished.

Left Atrium

Interruption of the inotropic response in the LA to stimulation of the right stellate cardiac nerve and the recurrent cardiac nerve was accomplished by transection of the SVC on all occasions. The response to VMCN stimulation was interrupted by transection of the superior LA in all experiments. The VLCN response in the LA was partially interrupted by transection of the superior LA, but usually required transection of the VLCN at the left superior pulmonary vein for total ablation of inotropic response in the LA. The inotropic response in the LA to stimulation of the right and left ansae was interrupted by a combination of transection of the superior LA and by interruption of nerves along the root of the great vessels. On one occasion, the response to stimulation of the right posterior ansa and left anterior ansa was not completely interrupted until transection of the inferior LA was accomplished.

In Figure 4 electrical stimulation of the left posterior ansa results in augmentation of contractile force in the RA, the LA (RAF and LAF) and in the RV (RVF) associated with cardio-acceleration. Following transection of the superior margin of the LA (column-superior LA), the augmentation of contractile force in the RA and LA no longer occurs, while augmentation in the RV as well as the cardio-acceleration continues. The sympathetic fibers from this nerve reach both the RA and LA by

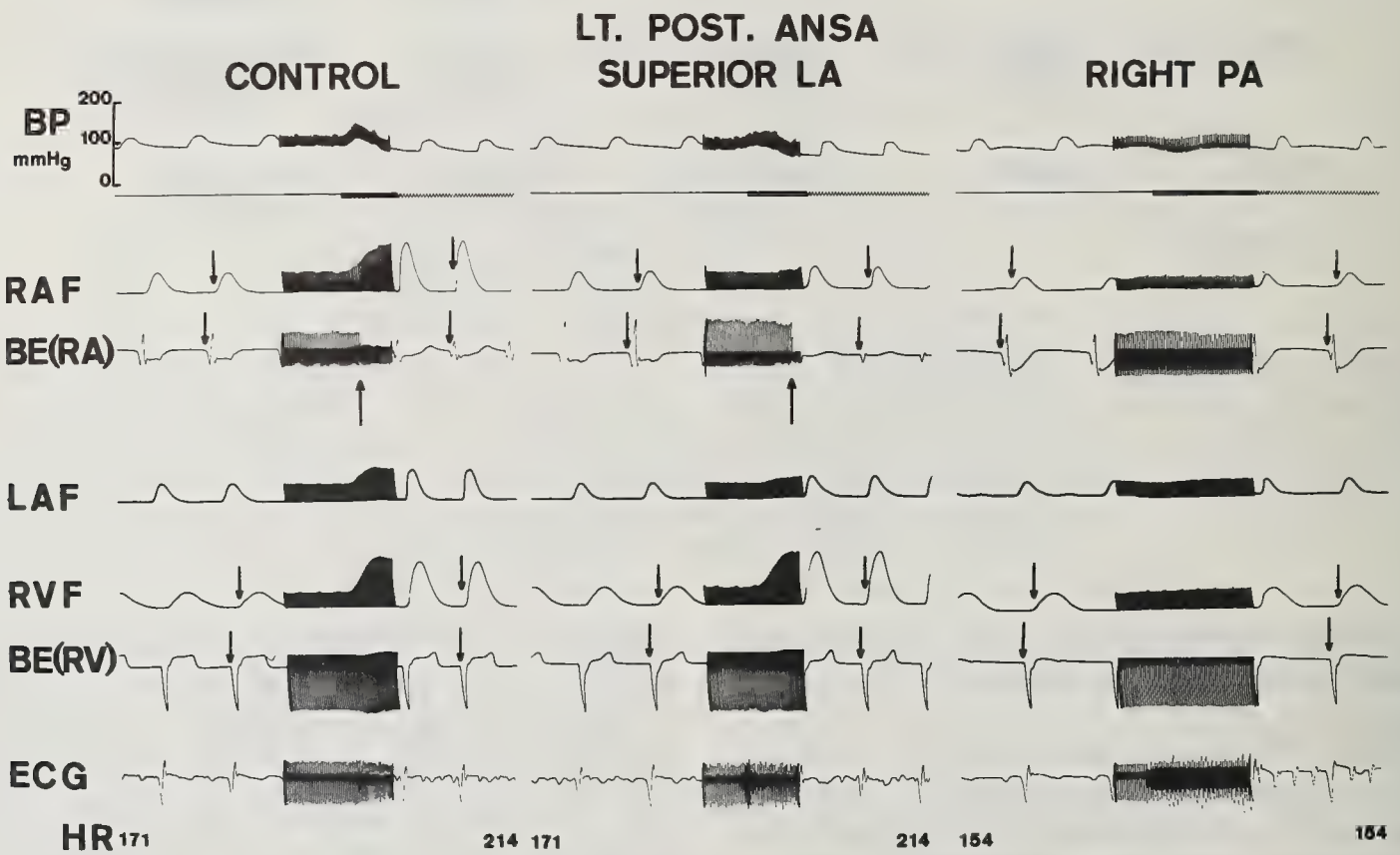


FIGURE 4.—*Regional Denervation of the Canine Heart.*
Lt. Posterior Ansa Stimulation
BP=blood pressure
RAF=rt. atrial contractile force
BE(RA)=bipolar electrogram, rt. atrium
LAF=lt. atrial contractile force
RVF=rt. ventricular contractile force
BE(RV)=bipolar electrogram, rt. ventricle

entering the heart along the superior margin of the LA while the cardio-accelerator fibers and those to the RV enter the heart along other routes.

Atrial Innervation: Parasympathetic

In all experiments a decrement in contractility occurred in both atria during electrical stimulation of the right and left thoracic vagus nerves, the right and left cervical vagosympathetic trunks, the craniovagal nerve and the caudovagal nerve. Stimulation of the recurrent cardiac nerve resulted in depression of RA and LA contractility in 11 of 17 experiments, while stimulation of the innominate nerve caused the response in the RA in 5 animals and in the LA in 2 animals.

Right Atrium

The parasympathetic response in the RA was interrupted in all experiments to stimulation of the left thoracic vagus, left cervical vagosympathetic trunk, craniovagal nerve, recurrent cardiac nerve and innominate nerve following a combination of two procedures: transection of the SVC and transection of nerves along the root of the great vessels. In the majority of experiments either intervention resulted in depression of the magnitude of response, but only rarely did either of the interventions completely abolish the response. Interruption of the parasympathetic response in the RA to stimulation of the right cervical vagosympathetic trunk and caudovagal nerve also required transection of the nerves along the root of the great vessels and transection of the SVC; however, in 3 experiments, dissection of the inter-atrial groove was necessary to completely abolish the response to these two nerves. The response to the right thoracic vagus nerve stimulation was not abolished by transections along the root of the great vessels. In contrast, the response was abolished in the majority of experiments by a combination of transection of the SVC and dissection of the inter-atrial groove. In 3 experiments, the parasympathetic response persisted until transection of the inferior margin of the LA was performed.

Left Atrium

The parasympathetic response in the LA during stimulation of the recurrent cardiac nerve was on all occasions interrupted after transection of the SVC whereas the response to innominate nerve stimulation was interrupted after transection of the superior LA. The response to stimulation of the right cervical vagosympathetic trunk and the craniovagal nerve was also interrupted following transection of the SVC in the majority of experiments. However, on two occasions, the response was not totally abolished to right vagosympathetic trunk stimulation until transection of nerves at the root of the great vessels was performed; in one experiment, the response to craniovagal nerve stimulation was totally abolished following transection along the great vessels. The response to left cervical vagosympathetic trunk and left thoracic vagus nerve stimulation was abolished following transection of the superior LA in the majority of instances. In two experiments, transection at the root of the great vessels was necessary to completely abolish the response to left vagosympathetic trunk stimulation, and in one experiment the same procedure was necessary to interrupt the response to left thoracic vagus nerve stimulation. Interruption of the parasympathetic response in the LA to caudovagal nerve and right thoracic vagus nerve stimulation required both transection of the SVC and transection of the inferior margin of the LA. Moreover, in one experiment, transection at the root of the great vessels was also required to totally interrupt the response to caudovagal nerve stimulation.

Note in Figure 3 that following left SVC transection, RA and LA contractile force (RAF and LAF) continue to be depressed during stimulation of the left cervical vagus nerve. However, following transection of the right SVC (column-right SVC), RA contractile force is no longer depressed, while LAF continues to be depressed with stimulation. After transection along the pulmonary artery (column-left PA), stimulation no longer effects depression of LAF. Thus, parasympathetic fibers from this nerve to the RA enter the heart along the right side of the SVC, while parasympathetic

fibers to the LA enter along the left side of the main pulmonary artery.

AV Node Innervation

Prior to surgical intervention, electrical stimulation of each of the cardiac nerves resulted in either sinus acceleration or a decrement in sinus rate in the majority of experiments (see sinus node innervation). Since the majority of sympathetic and parasympathetic nerve pathways to the sinus node enter the heart along the SVC, AV nodal rhythm was demonstrated in the eleven experiments in which transection of the SVC was the first intervention performed. Following transection of the SVC, acceleration of the AV node or slowing of the AV node was demonstrated during stimulation independent of neural influence on the sinus node. Moreover, in the few experiments in which sinus node responses persisted after SVC transection, neural influences upon the AV node were not demonstrated until the remainder of sinus node innervation was interrupted (see *Sinus Node Innervation*).

Sympathetic Innervation

Following interruption of sympathetic pathways to the sinus node, AV junctional tachycardia was demonstrated in all eleven animals. Average rates were 199/minute (range = 170 to 240). LAA and VLCN stimulation resulted in AV tachycardia in 11 animals, while LPA did so in 8 experiments, RAA in 7, and RPA in 6 experiments. Stimulation of the innominate nerve, recurrent cardiac nerve and craniovagal nerve each produced the response in 2 experiments, whereas VMCN resulted in AV nodal rhythm in 3 experiments. The caudovagal nerve and right thoracic vagus nerves each elicited the response in one of the eleven animals.

The AV nodal response to electrical stimulation of the caudovagal nerve, right thoracic vagus nerve, and innominate nerve, was interrupted after transection of the inferior margin of the LA on all occasions, whereas the response to stimulation of the recurrent cardiac nerve, VMCN and craniovagal nerve was interrupted following transection of nerves at the root of the great vessels in all cases. The RAA, RPA and LPA each exhibited a bimodal distribution to the AV node. Transection of nerves at the root of the great vessels interrupted the AV nodal tachycardia to RAA and RPA stimulation in 2 experiments, and to LPA stimulation in 4 experiments. Further abolition of the AV response occurred after transection of the inferior LA to LPA and RPA stimulation in 4 experiments, while the response to RAA stimulation was interrupted by this procedure on 5 occasions. The AV nodal response to LAA stimulation was interrupted following transection of the inferior LA in 6 experiments. In 3 additional experiments, transection of the nerves at the root of the great vessels was necessary for complete abolition of the response. In the remaining 2 experiments, the response was not interrupted until transection of the VLCN was performed. The AV nodal response to stimulation of the VLCN was interrupted after transection of the inferior LA in 7 experiments. In the remaining 4 experiments, both transection of the nerves at the root of the great vessels and transection of the VLCN were required to completely interrupt the response. Following either of the two latter procedures, the magnitude of the responses was usually decreased, however both procedures were necessary in each instance in order to result in interruption of AV nodal tachycardia.

Figure 5 depicts cardio-acceleration during electrical stimulation of the right anterior ansa from 146 to 250/minute (column-control). Electrical and mechanical activation of the RA (BE [RA] and RAF) precedes electrical and mechanical activation of the RV (BE [RV] and RVF). Following transection of the SVC (column-SVC transection), electrical stimulation results in increase in HR to 190/minute with electrical and mechanical activation of the RA and RV occurring simultaneously. Thus, the sympathetic fibers from this nerve to the sinus node enter the heart along the SVC and interruption of these neural elements unmasks AV junctional tachycardia. The sympathetic fibers to the AV node are interrupted following transection at the junction of the inferior vena cava with the inferior margin of the LA (column-IVC-ILA junction). Note that in the right

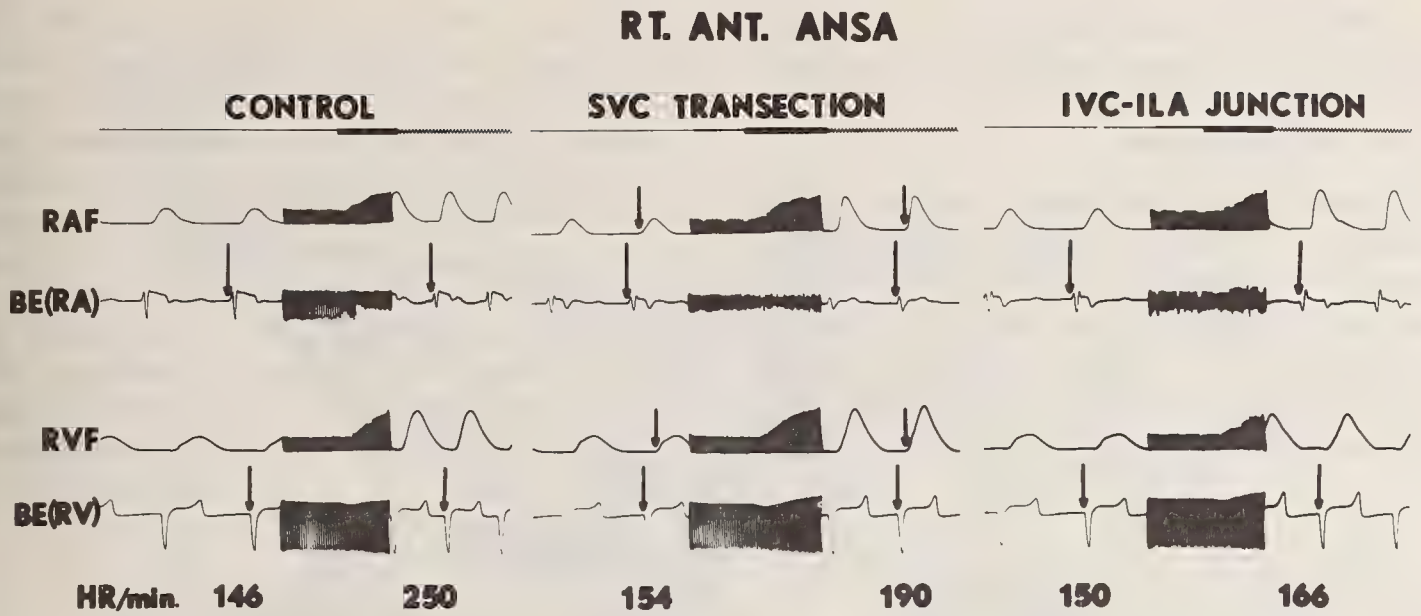


FIGURE 5.—*Regional Denervation of the Canine Heart.*
Rt. anterior Ansa Stimulation
RAF=rt. atrial contractile force
BE(RA)=bipolar electrogram, rt. atrium
RVF=rt. ventricular contractile force
BE(RV)=bipolar electrogram, rt. ventricle

column, stimulation results in slight cardioacceleration, but electrical and mechanical activation of the RA continues to occur 70 msec prior to that of the RV.

Parasympathetic Innervation

Parasympathetic innervation of the AV node was demonstrated in all animals in which transection of the SVC was the first procedure performed. Following effective parasympathetic denervation of the sinus node, stimulation of parasympathetic nerves results in slowing or cessation of ventricular rate accompanied by no change in atrial rate. In all eleven animals, AV nodal innervation was demonstrated during stimulation of the right thoracic vagus nerve, right vagosympathetic trunk, left thoracic vagus nerve and left vagosympathetic trunk. AV nodal parasympathetic innervation was demonstrated during stimulation of the caudovagal nerve in four experiments, during stimulation of the craniovagal nerve in two experiments and during stimulation of the recurrent cardiac nerve in one experiment. The remaining cardiac nerves did not provide evidence for parasympathetic innervation of the AV node in any experiment.

In all experiments the AV nodal parasympathetic response to stimulation of each nerve was interrupted following either transection of the superior margin of LA or transection of the inferior margin of the LA. Total interruption of depression of the AV node to nerve stimulation was accomplished by transection of the superior LA to right thoracic vagus nerve stimulation in 3 experiments, to right cervical vagosympathetic trunk stimulation in 2 experiments, to left cervical vagosympathetic nerve stimulation in 5 experiments, in one experiment each to left thoracic vagus nerve, craniovagal nerve and caudovagal nerve stimulation. In many experiments, transection of the superior LA did not totally interrupt slowing of the AV node during nerve stimulation. In these experiments, transection of the inferior LA invariably interrupted the AV nodal response to stimulation of each of the nerves. The left thoracic vagus nerve response was interrupted in 10 experiments, the right thoracic vagus response was interrupted in 8 experiments, the right cervical vagosympathetic trunk response in 9 experiments, the left cervical vagosympathetic trunk

in 5, the craniovagal, caudovagal and recurrent cardiac nerve response each in one experiment.

Ventricular Innervation

Evaluation of ventricular sympathetic innervation reiterates previous studies which have demonstrated that these nerves enter the heart along the great vessels and through the VLCN which enters the heart at the left superior pulmonary vein (see DISCUSSION). Figure 6 demonstrates augmentation in contractility in RV sinus (RVS-F), the RV outflow tract (RVO-F), the LV base (LVB) and the LV apex (LVA) during electrical stimulation of the left anterior ansa. These responses continue to be observed after transection of the entire atria, although rhythm shifts to AV junctional. However, following transection of nerves along the pulmonary artery and aorta (column-PA and aorta), none of the responses occur with neural stimulation. The sympathetic innervation to each of these areas of myocardium as well as to the AV node, there-

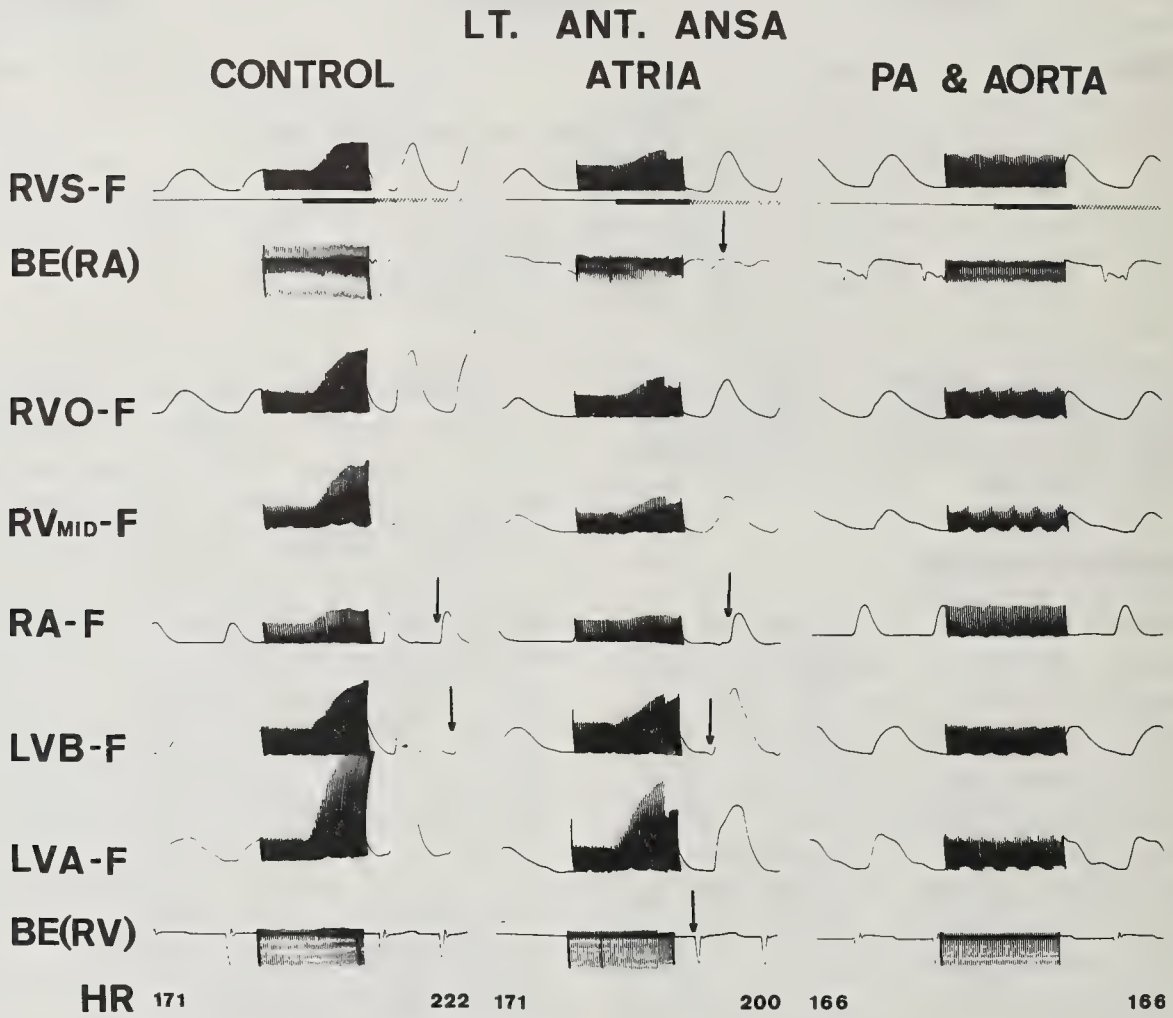


FIGURE 6.—*Regional Denervation of the Canine Heart.*
Lt. Anterior Ansa Stimulation
RVS=rt. ventricular sinus contractile force
BE(RA)=bipolar electrogram; rt. atrium
RVO-F=rt. ventricular outflow tract contractile force
RVMID-F=mid-portion rt. ventricle contractile force
RA-F=rt. atrial contractile force
LVB-F=lt. ventricular base contractile force
LVA-F=lt. ventricular apex contractile force
BE(RV)=bipolar electrogram, rt. ventricle

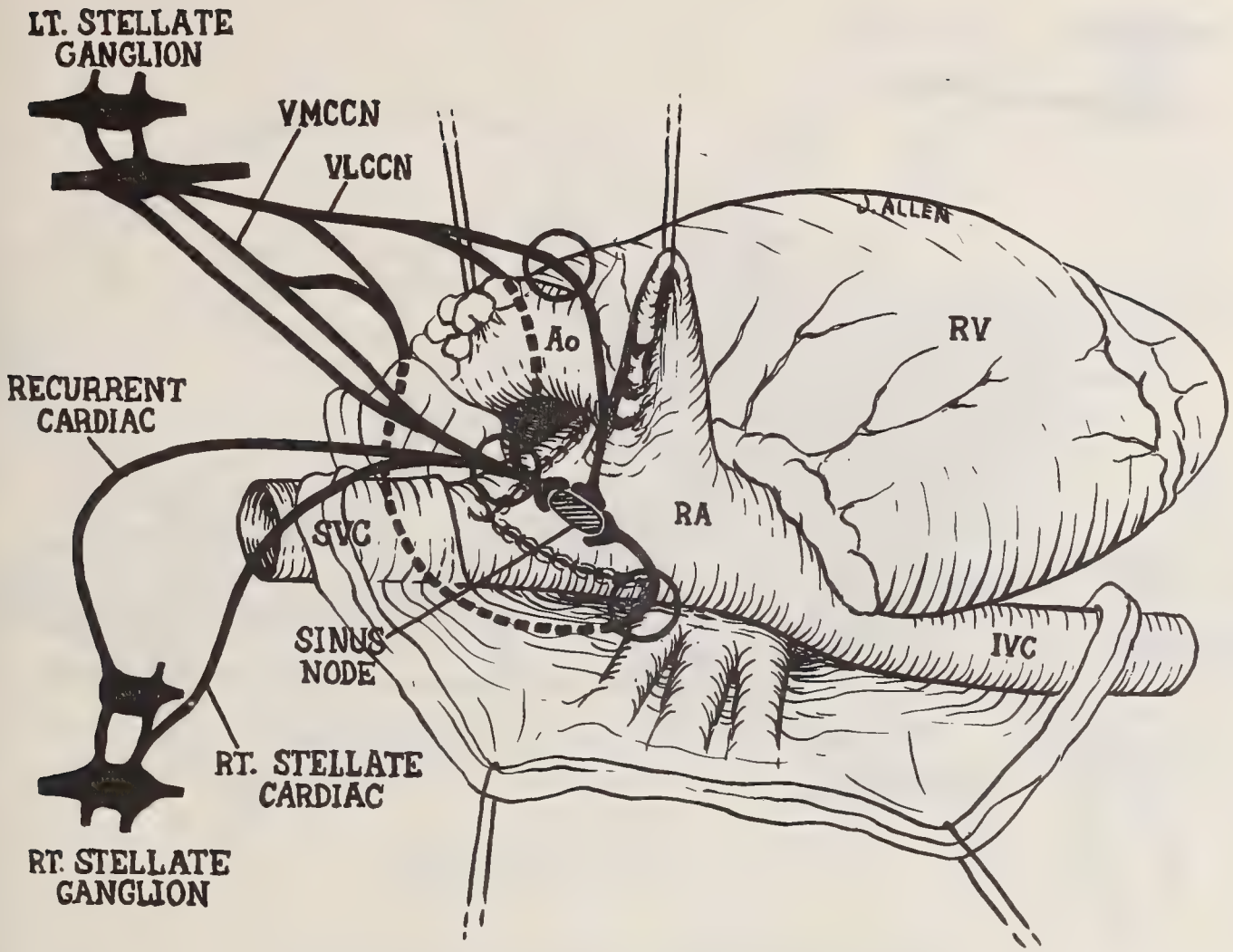


FIGURE 7.—*Regional Denervation of the Canine Heart.*
Rt. Lateral View of Heart
sympathetic innervation of sinus node

fore, enter the heart subjacent to the adventitia of the great vessels.

DISCUSSION

Sites of Entry of Neural Elements

Mizeres has anatomically described the only autonomic nerve which is large enough to trace to the heart: the VLCN.[3] This nerve reaches the pericardium as it descends anterior to the left pulmonary artery and left superior pulmonary vein. As it penetrates the pericardium, it branches profusely over the left wall of the left atrium. Randall has physiologically determined the VLCN to be purely sympathetic and that it innervates the left atrium and the lateral and posterior walls of the left ventricle.[4] Kaye has reported the other major sympathetic site of neural entry to both ventricles to be subjacent to the adventitia of the aorta and pulmonary arteries.[5] These nerve pathways innervate the anterior walls of both ventricles and travel from base to apex.[7] These investigators have recently demonstrated that the nerves entering along the great vessels also innervate the upper portion of the interventricular septum.[8] Moreover, they have delineated a third site of entry of sympathetic nerves to the ventricles. A group of nerves descend posterior to the heart and enter the heart in the oblique sinus at the junction of the inferior vena cava with the left atrium. These nerves provide sympathetic innervation to the posterior walls of both ventricles and to the interventricular septum as well as parasympathetic and sympa-

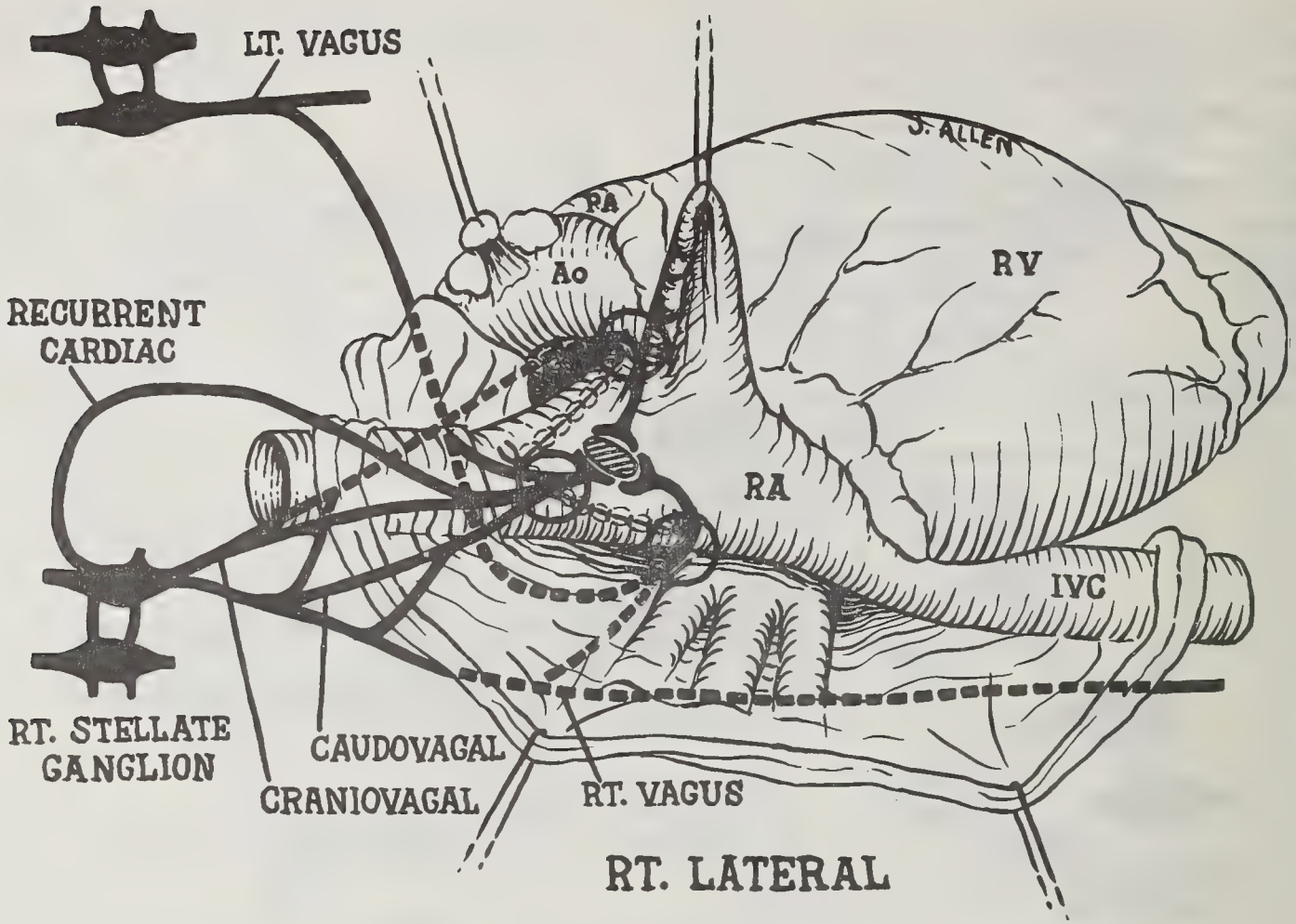


FIGURE 8.—*Regional Denervation of the Canine Heart.*
Rt. Lateral View of Heart
parasympathetic innervation of sinus node

thetic innervation to the AV node. Our data confirm these findings as well as delineate a second site of entry of sympathetic and parasympathetic innervation of the AV node. Sympathetic fibers of right-sided origin (Figure 9) reach the AV node from the junction of the inferior vena cava with the left atrium through the caudovagal nerve and occasionally the right thoracic vagus nerves. Right-sided sympathetic fibers reach the AV node along the great vessels through the recurrent cardiac and craniovagal nerves. Sympathetic fibers of left-sided origin reach the AV node through three sites of entry (Figure 10). The VLCN sends fibers along the left atrium to the AV node. The innominate nerve passes posterior to the heart and reaches the AV node by entering at the junction of the inferior vena cava with the left atrium. Both the VLCN and VMCN send fibers along the great vessels to reach the AV node.

The parasympathetic fibers of right and left-sided origin enter the heart in two areas: at the junction of the inferior vena cava with the left atrium, and along the superior margin of the left atrium presumably from the pretracheal plexus (Figures 11 and 12). Of left-sided origin, the left thoracic vagus sends parasympathetic fibers to the AV node both along the junction of the inferior vena cava with the left atrium and at the superior margin of the left atrium (Figure 11). Parasympathetic fibers of right-sided origin reach the AV node through the craniovagal and caudovagal nerves as well as through the right thoracic vagus nerve by entering at the superior margin of the left atrium, while fibers from the recur-

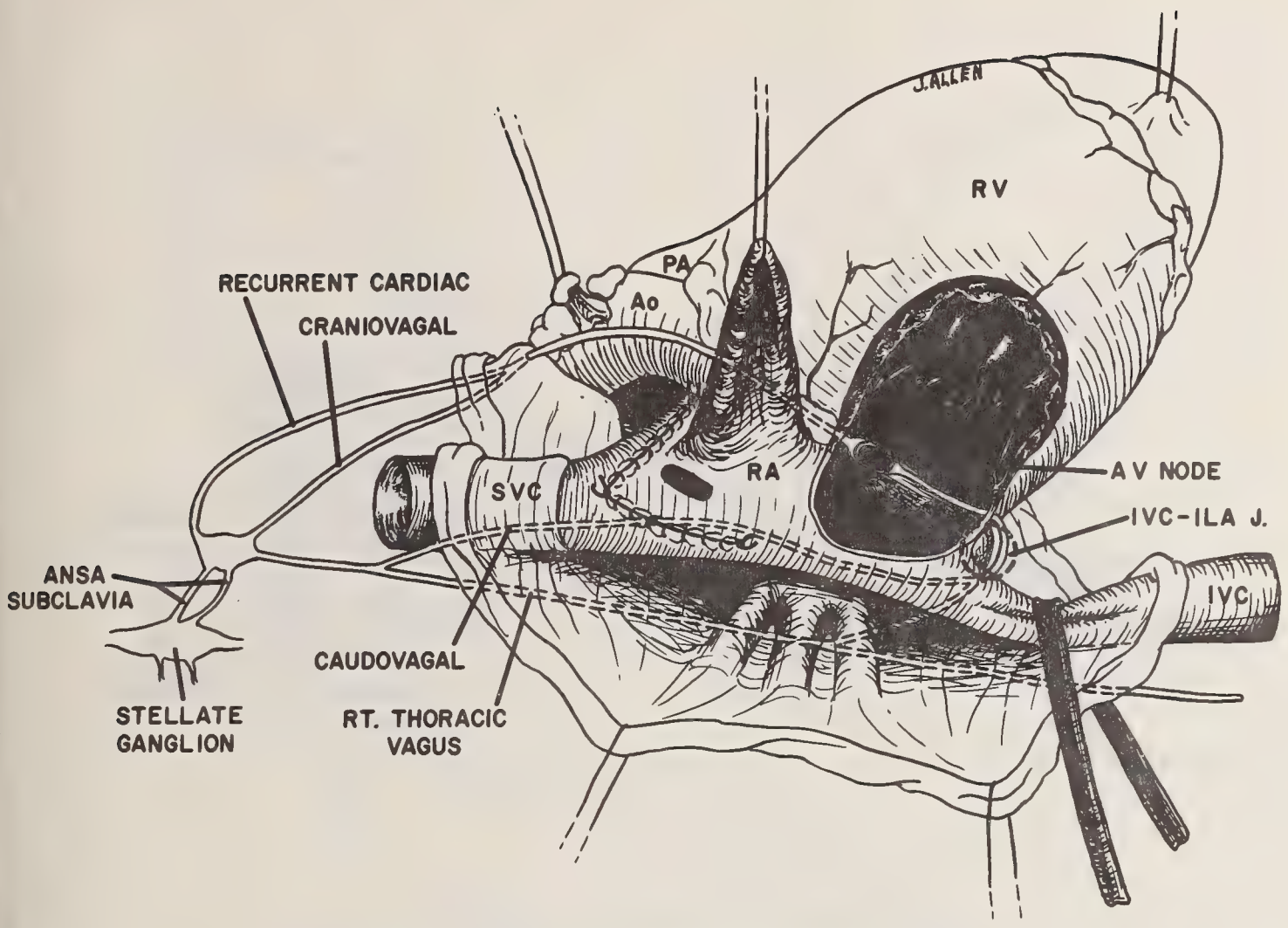


FIGURE 9.—*Regional Denervation of the Canine Heart.*
Rt. Lateral View of Heart
sympathetic innervation of AV node, rt.-sided origin

rent cardiac nerve, the caudovagal nerve and the right thoracic vagus nerve enter at the junction of the inferior vena cava with the left atrium (Figure 12).

Sympathetic innervation of the sinus node enters the heart along two pathways (Figure 7). The recurrent cardiac nerve, right stellate cardiac nerve, VMCN, and VLCN all send sympathetic fibers along the intra-pericardial SVC to the sinus node. The VLCN and the VMCN also send fibers subjacent to the adventitia of the aorta and pulmonary artery to reach the sinus node. Parasympathetic fibers to the sinus node enter the heart along the superior margin of the LA, the SVC, and the inter-atrial groove. The left vagus nerve, the craniovagal nerve, the caudovagal nerve and the recurrent cardiac nerve all send fibers along the SVC. Both the left and right thoracic vagus nerves send fibers along the inter-atrial groove, while the craniovagal nerve also sends fibers to the sinus node via the superior margin of the LA (Figure 8).

SUMMARY

These data demonstrate the sites of entry of autonomic cardiac nerves to the heart. The physiological consequences of interruption of these neural pathways have been discussed. Awareness of the pathways of these neural elements must be considered when the technical aspects of cardiac manipulation and implantation of recording devices are undertaken.

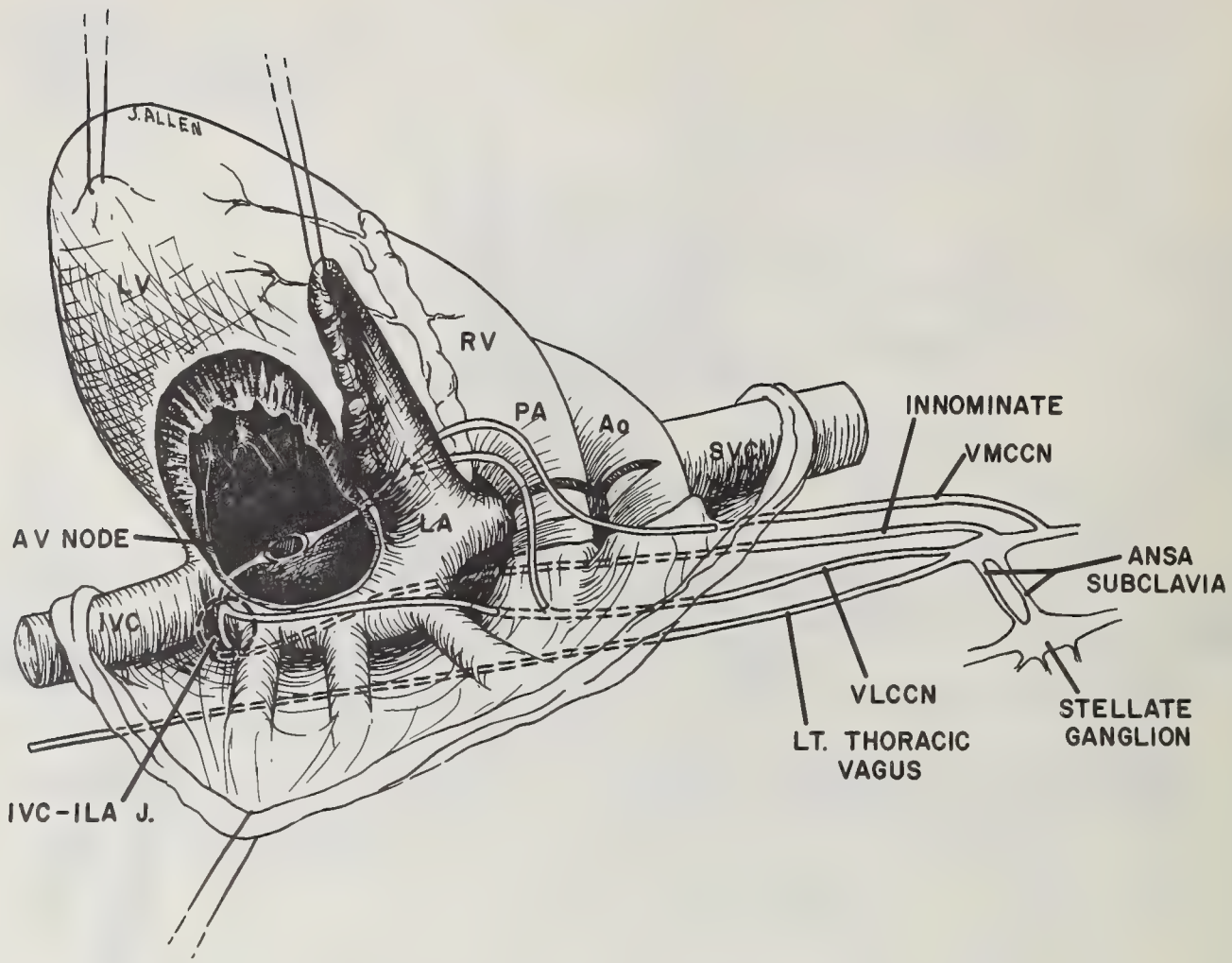


FIGURE 10.—*Regional Denervation of the Canine Heart.*
Lt. Lateral View of Heart
sympathetic innervation of AV node; lt.-sided origin

REFERENCES

1. PEISS, C. N. and MANNING, J. W. Effects of sodium pentobarbitol on electrical and reflex activation of the cardiovascular system. Circ. Res. 14:228–235, 1964.
2. DANIEL, E. E., FULTON, J. B., HIDDLESTON, M., MARTIN, W. and FOULKS, J. G. An analysis of the mechanism of barbiturate induced cardiovascular depression and its antagonism by sympathomimetic amines. Arch. Intern. Pharmacodyn. 108:457–472, 1956.
3. MIZERES, N. J. The anatomy of the autonomic nervous system in the dog. Am. J. Anat. 96:285–318, 1955.
4. SZENTIVANYI, M., PACE, J. B., WECHSLER, J. S. and RANDALL, W. C. Localized myocardial responses to stimulation of cardiac sympathetic nerves. Circ. Res. 21:691–702, 1967.
5. KAYE, M. P., GEESBREGHT, J. M. and RANDALL, W. C. Distribution of autonomic nerves to the canine heart. Am. J. Physiol. 218:1025–1029, 1970.
6. PACE, J. B., GEIS, W. P., KAYE, M. P. and PRIOLA, D. V. Influence of ventricular epicardiectomy on cardiac responses to stellate ganglion stimulation. J. Thoracic and Cardiovas. Surg. 58:39–47, 1969.
7. GEIS, W. P. and KAYE, M. P. Distribution of sympathetic fibers in the left ventricular epicardial plexus of the dog. Circ. Res. 23:165–170, 1968.
8. RANDALL, W. C., ARMOUR, J. A., GEIS, W. P., and LIPPINCOTT, D. B. Regional cardiac distribution of sympathetic nerves. Fed. Proc. 31:1199–1208, 1972.

DISCUSSION

KARL WEBER, University of Alabama, Birmingham, Ala.: Dr. Geis' eloquent paper is now open for discussion. I would like to reiterate Dr. Geis' comments about chronic instrumentation when implanting electromagnetic flow probes around the aorta, pulmonary artery, or left main coronary artery. We had best con-

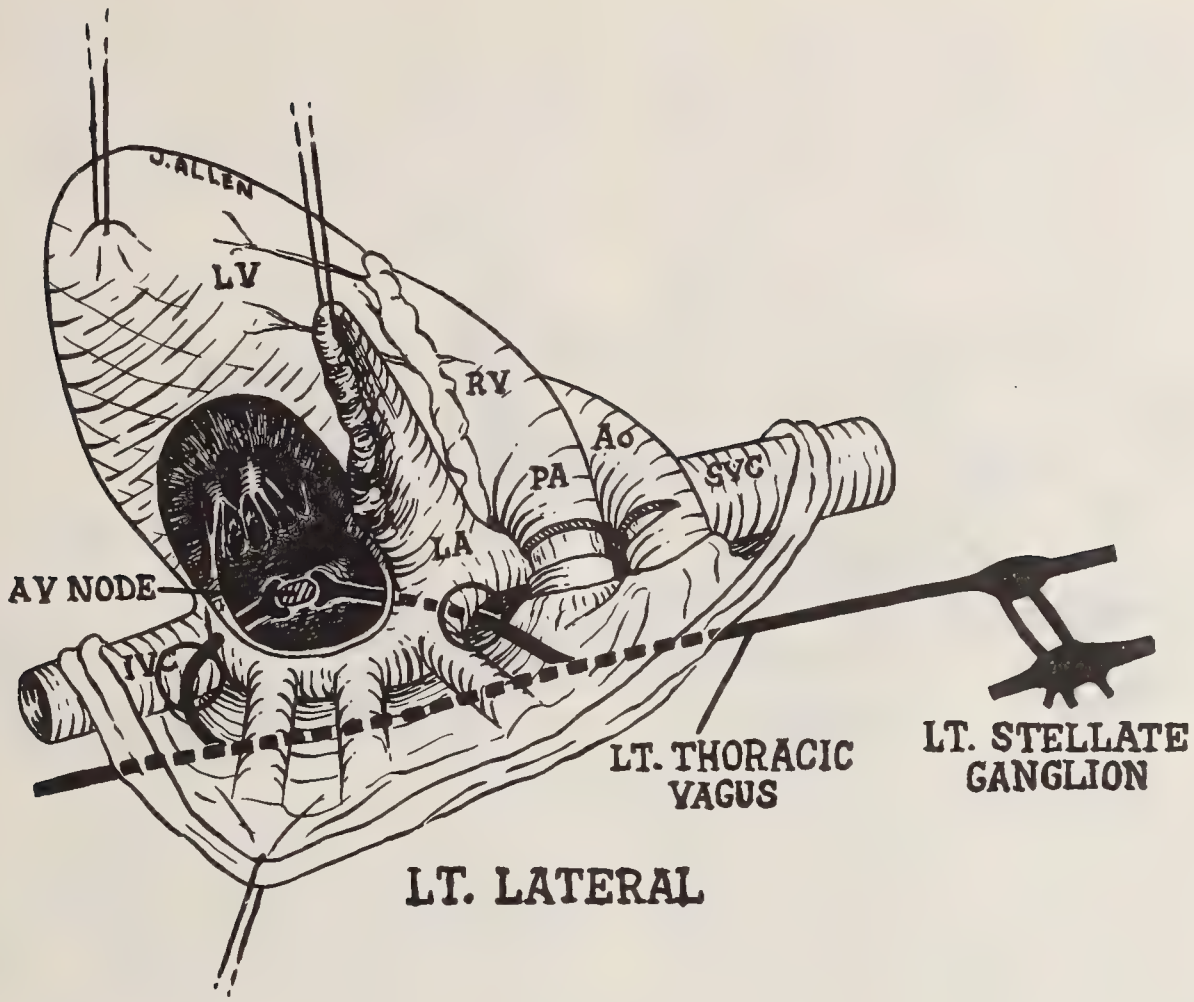


FIGURE 11.—*Regional Denervation of the Canine Heart.*
Lt. Lateral View of Heart
parasympathetic innervation of AV node, lt.-sided origin.

sider if we have a partially or totally denervated preparation.

CHAIRMAN M. P. KAYE: Thank you very much, Dr. Weber and Dr. Geis. We will take the next 5–10 minutes for Dr. Peters of the University of Utah to discuss briefly preconditioning, implantation, and postoperative care of calves with artificial hearts used at the University of Utah.

JEFFREY L. PETERS, University of Utah, Salt Lake City: I'd like to review briefly some of our experiences with more than 70 calves with total heart implants in the last 2 years and talk to you about our methodology.

First, we've tried to improve the quality of the calf prior to delivery to our facilities. In order to do this, we set up an extremely elaborate preconditioning program. Our calves were obtained from dairies in Utah and brought to a large facility that could hold 600–800 calves at one time. The facility had a nursery that held 300 baby calves in elevated pens who were fed a special diet of alfalfa pellets and milk substitutes.

They were then weaned and transferred to growing pens of cement with straw and flushing troughs, and these educated calves deficated and urinated in the troughs, not on the straw. These calves were immunized against calf diseases, and we had a double blood scan run on each before it was brought to our facility. When they arrived, we ran more blood scans and did respiratory function studies. Our calves were all one breed, Holstein, and all approximately

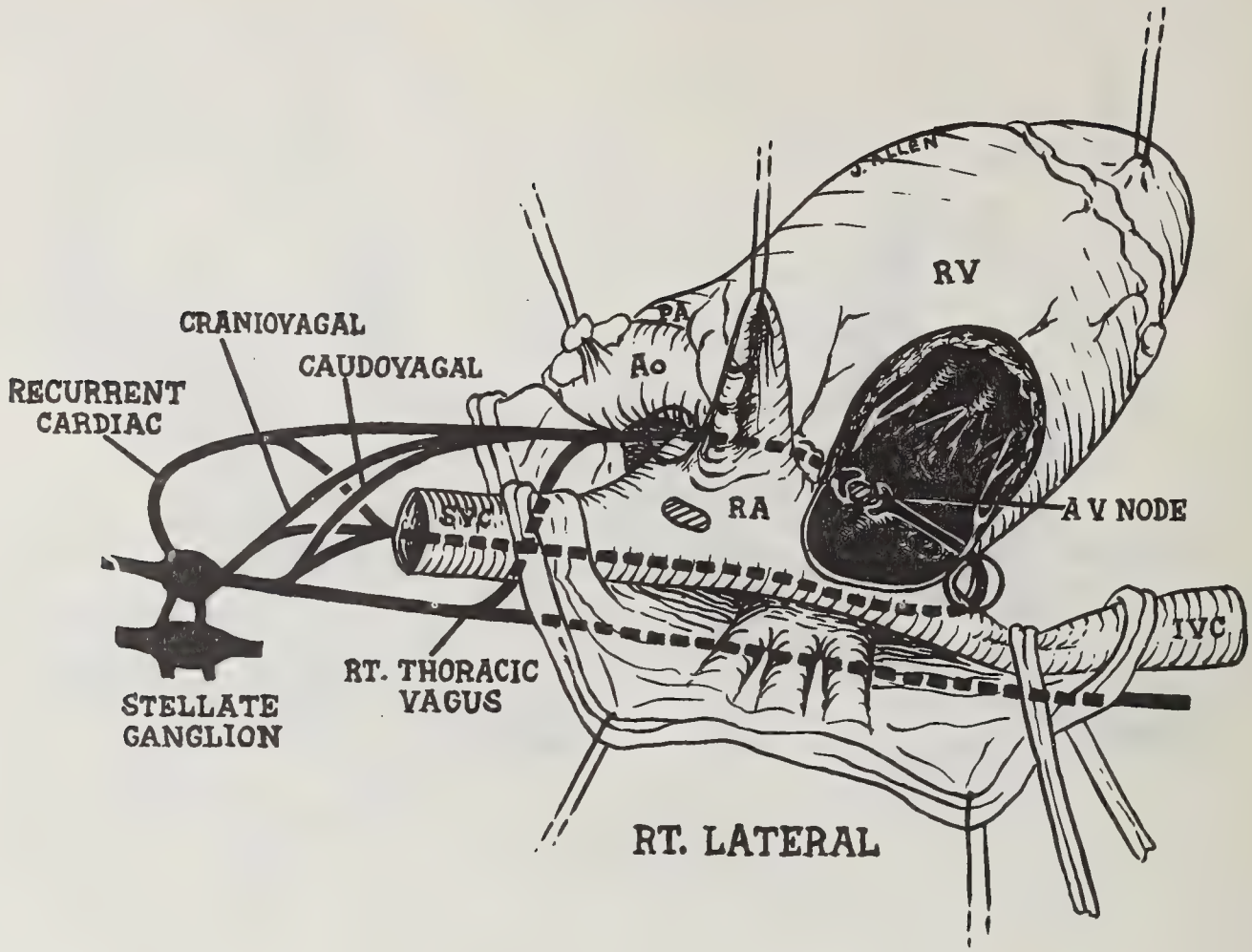


FIGURE 12.—*Regional Denervation of the Canine Heart.*
Rt. Lateral View of Heart
parasympathetic innervation of AV node; rt.-sided origin.

the same age and weight which standardized our procedure.

We next looked at the different implantation procedures we were using for different purposes. We used the traditional cardiopulmonary bypass, particularly to evaluate a new heart when we're not sure of the fit and we may need a lot of time to get it in. The most exciting procedure, introduced by Dr. Jun Kawai of Japan, is deep hypothermia. We've had our greatest success with implanting the heart under deep hypothermia.

If I may have some slides, I'll briefly show you. Before we put the heart in, we like to test it extensively. We have long-term and short-term testing. This is an artificial heart and the curve shows cardiac output and filling pressure. This next slide shows the Kwan-Gett hemisphere of the left heart. We have two sets of curves; the zero vacuum is here at the dotted lines, and on this curve we have a vacuum applied to the diaphragm. You can see how this shifts the curve from the right to the left. We feel it is better to do this because if you put a heart in and it doesn't work, you've lost a lot of money.

Next slide, please. This is a Donovan spherical heart that can pump up to about 14 liters, and you can see how the hearts differ. This is a different design with different characteristics. When we have this information, we can put the heart in, using fewer translucents.

May I have the next slide? This is a hematic of our most successful implantation procedure,

deep hypothermia, showing the changes in heart rate and blood pressure with temperature, as you cool the animal down in an ordinary bathtub. Of course the expense of this is very little—just some ice. You don't have any membrane oxygenator or the like, and you use very little anesthesic, because once the animal gets cool it isn't necessary. With this procedure, we can separate the effects of pumping of the artificial heart from those of implantation more accurately than with the other processes. I'm sure it's obvious to all of you that if you have hemolyzed blood as a result of cardiopulmonary bypass and any platelets in your system, it's going to be difficult to evaluate the materials and pumping of the artificial heart. We have circulatory and respiratory rests for a maximum of one hour. We average about 35 minutes with the artificial heart in, and then we just put the calf back in the bathtub and warm him up.

Next slide, please. This is a series of records. We want to evaluate the hemodynamic effects of the artificial heart so that we can then forget about our pressure transducers and look at other things. We do this first because we know that the more pressure transducers we have, the more disturbance and variables we will have in the system. This is just a typical system here where we have right atrial, right ventricular, pulmonary arterial, left atrial, and left ventricular aortic pressures, and you can see some of the tracings. This is with a Donovan spherical heart.

Next slide please. This is a typical blood pressure record for one of our long survivors with a total artificial heart. This will give you an idea of the parameters we monitor during the procedure.

May I have the slide off. Another method that we've used is double bypass, introduced by Dr. Kwan-Gett. We attach the artificial heart, leaving the natural heart in; when the artificial heart is pumping, we then remove the natural heart and sew up the chest. This method, like deep hypothermia, produces no hemolysis and maintains platelets and coagulation factors.

We next tried to improve our postoperative care procedures, which required extensive training of people to work in the Intensive Care Unit. We had two people with our calves at all times monitoring the animals, watching all the equipment, and recording extensive data.

Now let's quickly go through some of the data we got. One of our problems with the artificial heart is high central venous pressure. As you can see, here's an animal living for a long time and the pressure, which should be down around zero, is very high.

Next slide, please. Again we monitor respiratory function all the time. Here's an animal that lived for almost 11 days. Blood gas is rather normal. He was without any respiratory support for a period of one week, and then he required nasal oxygen and finally the respirator.

Next slide please. Here's another thing we look at—respiratory function. Dr. Donovan is now setting up extensive function tests of the lungs. We're measuring diffusion capacities. In most of our animals we find a decreasing functional resistive volume.

Next slide. Again, more data that we look at: blood volume, hematocrit, plasma volume, and cell volume. Frequently we have to give blood to a calf that has an artificial heart. One thing I'll point out is that blood volume in a calf with an artificial heart tends to decrease, giving us the symptoms of artificial right heart failure.

Next slide. Here's another thing we can monitor, the electrical atriogram rate. We monitor the electrical activity of the natural atria that is still there. We actually use this electricity to drive the artificial heart. Last slide. It is important to look at the fit of the hearts in the animals. We just suspend the animal, remove the chest, and take the lungs out, and we can then evaluate the hearts and set up new design criteria.

CHAIRMAN: Thank you Dr. Peters. I'm sure that you'll be available if there are any questions.

SURGICAL EXPERIENCE WITH NONHUMAN PRIMATE ANIMALS 1963-1971

E. I. Goldsmith*

In the period 1963–1971, nonhuman primate animals have been used in an increasing number and variety of experimental surgical procedures. Problems of husbandry, anesthesia, tranquilization, physical control while awake, prevention of disease transmission, fluid and electrolyte balance and postoperative care have now been solved enabling one to make a choice of primate animal subject based solely on the scientific merit of the species available. Chimpanzees *(Pan troglodytes)* are most similar to the human in size and physiologic and immunologic parameters but they are scarce, expensive and threatened with extinction. Baboons *(Papio sp.)* are the most valuable "general use" primates for medical and surgical research. They are larger than other monkeys, hardy, and adapt well to laboratory conditions. Although purchase price is approximately twice that of a dog, many investigators have found that productivity is greater and that total costs are reduced by using baboons. Rhesus monkeys *(Macaca mulatta)* are still the most widely used primates because they are plentiful and the large background of accumulated information is very valuable. Studies in reproductive and perinatal physiology are widely performed in rhesus monkeys because they can be bred with relative economy. During the last five years we have had experience with animals of 5 species in 30 different surgical procedures. The specific advantages of species for each type of experimentation will be demonstrated.

INTRODUCTION

It is significant that the experimental laboratory in most departments of surgery is known as the "dog lab". The experience and training of most investigators in surgical disciplines fosters the assumption that one should use the dog as the most appropriate animal for experimental surgical procedures. This assumption stems from a time when stray dogs were readily available and very inexpensive and when other animals were not available, or were more expensive. It neglects the now well established fact that canine anatomy and physiology are, in many cases, poor models for the human and that husbandry and handling methods have made better models, notably nonhuman primates, widely available and relatively inexpensive.

* Department of Surgery, New York Hospital-Cornell Medical Center, New York, N. Y., Laboratory for Experimental Medicine & Surgery in Primates (LEMSIP), New York University Medical Center, New York, N. Y.

METHODOLOGY

Because it is obvious that the ultimate limiting factor in the success of clinical kidney transplant programs is the source of organs for donation, we investigated the logistic and procedural problems surrounding the use of nonhuman primate animals as organ donors as well as the immunologic responses governing xenografts. In 1964, we performed two transplants from chimpanzees to patients for whom no human donor could be found. We observed excellent renal function in these transplanted kidneys followed by reversible acute rejection episodes not unlike those observed in human allografts. This experience convinced us that xenografting of organs would be a reasonable long term goal, and we undertook a program for the study of nonhuman primate animals in relation to man. This was ultimately enunciated in the editorial in the first issue of the *Journal of Medical Primatology*[1] where J. Moor-Jankowski and I described "medical primatology" as the "interface between human and veterinary medicine aided by classical primatology and zoology as well as anthropology and related sciences."

In 1965, with B. H. Kean, I infected two baboons with cercariae of *Schistosoma mansoni* and subsequently demonstrated that the resulting severe clinical infections could be cured by extracorporeal filtration of the portal vein blood.[2] The procedure was subsequently introduced into clinical practice and is now being used in selected cases throughout the world.

Since 1966, we and others have been investigating the efficiency of heterologous cross cir-

culation between nonhuman primates and human patients in hepatic failure. Results of the small experience which has been reported so far show that the procedure is practical and that there is some reason to believe that temporary augmentation of liver function is obtained.[3]

These experiences form the background for a personal conviction, that nonhuman primates must be the "animals of choice" for surgical research in the future whenever close approximation of human structure and function are required. Experiences of other investigators who have reached similar conclusions are reported in the proceedings of the First and Second Conference on Experimental Medicine and Surgery in Primates which were published in 1969 and 1971.[4,5] Both conferences included experimental surgical reports, some of which are discussed below. This is not to say that other animals will not continue to be important, but it does emphasize the vital necessity for access to primates by sophisticated medical scientists.

In the past, investigators frequently accepted whatever primates were available and often did not differentiate among "monkeys." Furthermore, the husbandry methods of traditional veterinary practice did not account for the transmissibility of infectious agents between human and nonhuman primates, with the result that many primate colonies were decimated by tuberculosis and other respiratory and gastrointestinal infections from human contacts. The reverse problem posed by transmission of herpes infections (monkey B virus), innocuous in the host animal but producing high mortality rates in humans and, more recently, the Marburg Disease epidemic in Germany induced fear of primates among many scientists. The difficulties and physical dangers of handling these animals were also recognized as deterrents to their use; and, finally, the cost of nonhuman primate animals was thought to be very high.

In 1966, the Committee of Scientists for the Use of Primates in Medical Research promulgated the following statement:

> We have in common a conviction that our scientific research has benefited from or has been dependent upon. the use of primate animals.

Representing many disciplines, each of us has turned to primates in investigative endeavors because of the opportunities offered by these animals to come closer to the conditions and the challenges offered by human structure, human function and human disease.

In many cases, the use of primates has presented unusual technical difficulties, inconvenience, and expense, and has caused the development of highly specialized techniques and procedures. However, the indispensable information we have obtained has justified the effort.

We believe that our experience will soon be shared by others and that the importance of primates in medical research will grow rapidly. This will intensify an already existing scarcity of animals, facilities, and knowledgeable laboratory personnel.

We have, therefore, constituted ourselves a Committee of Scientists for the Use of Primates in Medical Research and agree to the pursuit of the following principles:

1. Their unique biological status and their scarcity separate primates from the more common laboratory animals.
2. There is a need for an ongoing and increasing supply of great apes as well as other primates and plans must be developed for breeding, housing and study.
3. Methods, systems, know-how, and techniques which are generally used in laboratory animal care are not necessarily appropriate for the primates.
4. Knowledge of primate anatomy, physiology, pathology, etc. is still fragmentary and urgently requires intensive study.
5. The animals available must be utilized in the most efficient manner that can be developed and the techniques and facilities necessary for their study should be shared by the scientific community.
6. The foregoing principles can be implemented by the creation of a Laboratory for Experimental Medicine and Surgery in Primates in the New York vicinity, and this laboratory should be

widely supported by the medical and lay community as a supplementary resource to other medical facilities.

7. The director of this laboratory should be a medical scientist whose research interest and experience with primates further qualify him for this post.

In the ensuing years, the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) has grown into a research resource sponsored by the Associated Medical Schools of New York and New Jersey and administered by New York University Medical Center. The experiences of our laboratory in organization, animal husbandry, disease control, and teaching have been published [6–10] and have led to the conclusion that problems of husbandry, physical control, anesthesia, tranquilization, prevention of disease transmission, fluid and electrolyte balance and postoperative care have been solved, enabling one to make a choice of primate animal subject based solely on the scientific merit of the species to be used.

High cost of experimentation with nonhuman primates has often been mentioned as a deterrent to their use. In this context, it is true that the initial purchase price of most primate animals is higher than that of a mongrel dog. However, in New York City and in other areas, mongrel dogs are no longer obtainable from humane societies as they were in past years and the dollar outlay for purchase from dealers is steadily rising in response to demand for higher standards of care and housing. Animals bred specifically for experimentation and maintained until maturity already approach and exceed the cost of imported primates.

The cost factor must be viewed in relation to the scientific productivity of the animal model and the value of wasted professional and technical time in performing experiments which do not ultimately have applicability to man. This concept has been well accepted in the fields of immunology, infectious disease, reproduction, behavior and neurological research. In physiological and surgical experimentation the similarities between dog and man have been overemphasized and the differences have been overlooked. Nevertheless, those involved in the study of lung transplantation,[11] biliary tract[12] and portal system[2,13] have clearly stated the need to abandon the dog and to use the primate (baboon) accepting the fact that costs per animal may be higher but that the cost for an entire study will be much lower and that more worthwhile information may be obtained.

Finally with increased efficiency in the husbandry of baboons tending to reduce maintenance costs while the purchase price of dogs has been rising, the actual dollar gap is closing. In the New York Hospital-Cornell Medical Center the per diem charge to investigators to house baboons is now $1.40 while dogs cost $1.75.[14] The purchase price for a conditioned mature mongrel dog (wormed, vaccinated against hepatitis and distemper and observed for two weeks) is $70. Fox hounds are obtainable for $200 and beagles may cost as much as $100. Young female baboons may be obtained for $130. The comparative cost is illustrated in Figure 1, which clearly shows that the expenditure becomes equal at one year and thereafter the baboon is actually cheaper. Thus for long term experiments the economic, as well as the scientific argument already favors the nonhuman primate.

We reported in 1969[15] the pattern of primate utilization as determined from the literature. During recent years at LEMSIP we have had surgical experience with 5 species and have observed their use by many investigators utilizing different surgical procedures. A summary of the experience for a typical recent year is found in Table I.

Surgery upon new world monkeys was limited to four testicular biopsies which were obtained for a phylogenetic study of spermatogenesis. The thyroid tumor removed from the gibbon was an anaplastic carcinoma discovered as an incidental finding on physical examination. The surgical experience with rhesus monkeys is related almost entirely to our large reproduction, perinatal growth and development program. For several reasons reflecting cost structure and the previous experience of participating investigators, the breeding females in the program are 45% baboons and 55% macaques. Thus the surgical experience with macaques relates largely to reproductive studies.

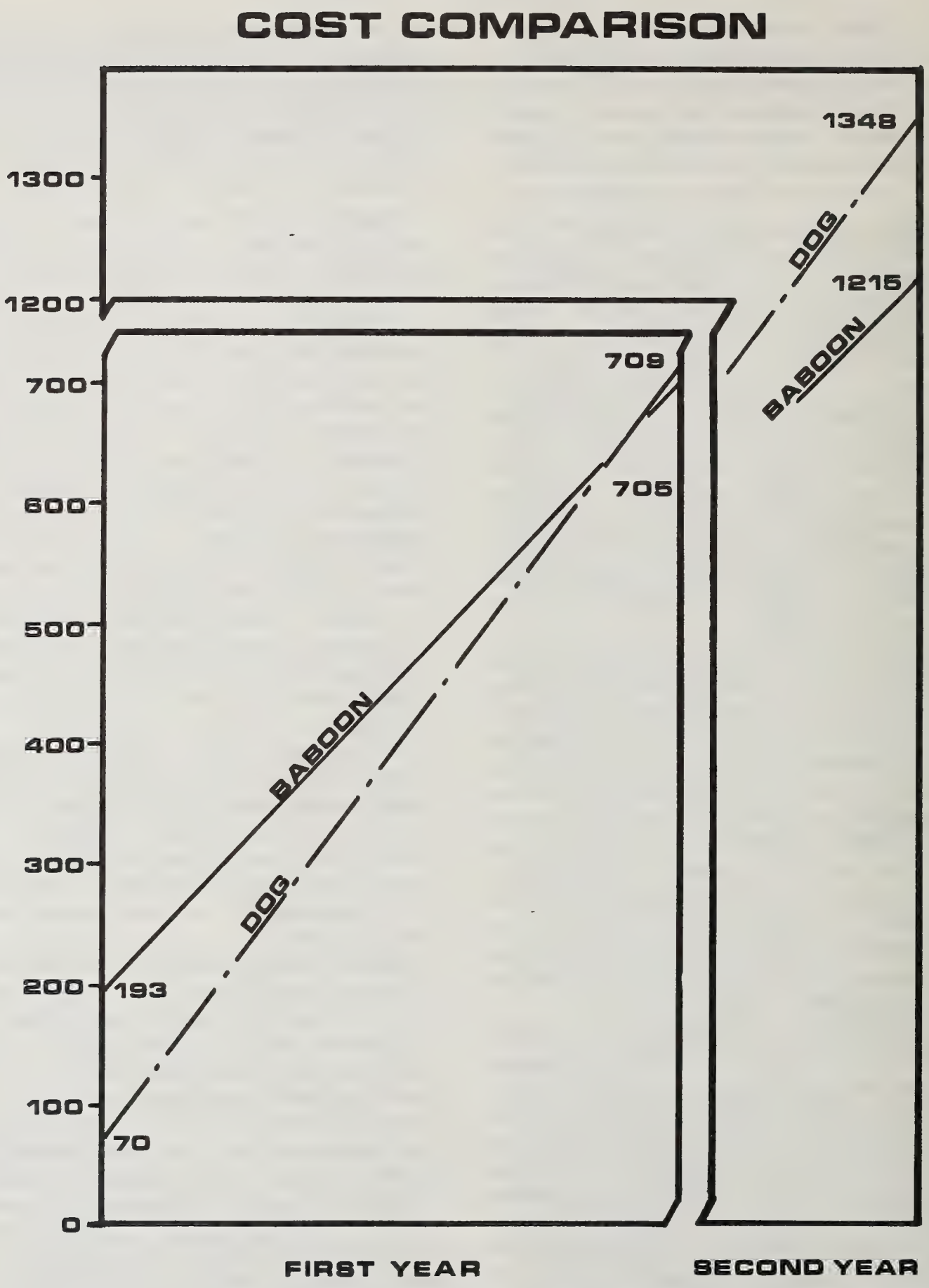


FIGURE 1.—Comparative Cost of Purchase and Utilization of "Conditioned" Mongrel Dogs Versus Conditioned Imported Adult Baboons For Long Term Experiment In A New York Medical School

TABLE I.—*Operative Experience For One Year at LEMSIP*

199 Operations	5 Species		
Chimpanzee *(Pan troglodytes)*	Liver biopsy	2	
	Heart biopsy	6	
	Kidney biopsy	7	
	FTSD	3	
			18
Gibbon *(Hylobates lar)*	Excision thyroid tumor	1	
			1
Baboon *(Papio cynocephalus)*	Liver biopsy	61	
	Caesarean section	1	
(Papio anubis)	Cannulation umbilical vein	6	
	Cannulation duodenum	2	
(Papio hamadryas)	Bile salt infusion	4	
	Cross-circulation with human	2	
(Papio papio)	Breast biopsy	1	
	Lens transplant	6	
	Corneal transplant	32	
	Autotransplantation of uterus	2	
	Tubal ligation	4	
	Extracorporeal hemofiltration of portal blood	3	
	Excision nasal septum	4	
	Prostoglandin induction of labor	5	
	Intestinal resection and reanastomosis	1	
	Exchange transfusion with human blood	3	
	In utero injection of fetus with radioactive isotype	2	
			139
Rhesus monkey *(Macaca mulatta)*	Caesarean section	27	
	Hysterectomy	2	
		2	
	Neonatal hypophysectomy	2	
	Bone biopsy	4	
			37
Squirrel monkey *(Saimiri sciureus)*	Testicular biopsy	4	
			4
			199

Surgical experience with chimpanzees has been largely limited to biopsy of organs actually or potentially damaged after experimental exposure of the animal to infectious agents which affect only chimpanzees and humans (hepatitis virus, streptococci). Previously, we had the opportunity to remove kidneys from chimpanzees for donation to human recipients. These experiences afforded us the opportunity to develop anesthesia techniques which, in fact, are exactly similar to those used for human patients, and to perform extensive exploration of abdominal and thoracic organs (which are eerily similar to the human). Attention to control of heat loss and maintenance of fluid and electrolyte balance are essential.

Seventy percent of the procedures were performed upon baboons reflecting the growing satisfaction of many investigators with the baboon as a general purpose animal model. The large number of liver biopsies stems from a large study of the effect of diet and alcohol upon liver morphology and function. The large group of corneal and lens transplants demonstrates opthalmologists' acceptance of the baboon eye as a suitable experimental model. Exchange transfusion with human blood and cross-circulation with human patients in hepatic failure is an interesting area of study which clearly shows the close phylogenetic relationship which cannot be duplicated with other animals. The recently recognized occurrence in baboons of gallstones with chemical composition similar to human gallstones has opened an entirely new area for investigation. Thus there are a growing

number of procedures relating to hepatic and biliary tract function.

There is a surprising absence of surgical procedures relating to heart and lung studies despite the past demonstrations that primates are excellent subjects for lung transplantation,[11] studies of atherosclerosis [16] and telemetry of free ranging animals.[17] We believe this to be a function of utilization patterns established in past years when the major thrust came from groups interested in immunology, reproduction and infectious disease. The value of allografting and xenografting of hearts in primates is clearly enhanced by the extensive knowledge of ABO blood groups of rhesus and other old world monkeys, baboons and chimpanzees which has been compiled by Moor-Jankowski and Wiener.[18] This information is not available for mongrel dogs. It should be emphasized that analogs and homologs of human blood groups have been identified in primates documenting their phylogenetic closeness to the human. This has enabled us to exchange transfuse animals with blood from different species for the purpose of cross-circulation and to study the immunologic consequences of this type of xenografting.[19]

In the 200 operations described, there have been four deaths. The gibbon developed tracheal collapse following excision of a malignant thyroid tumor and died during the first postoperative night. A baboon, which had an experimental biliary fistula draining via a T-tube and was trained to live in a chair for prolonged specimen collection, died ten days postoperatively of fluid and electrolyte depletion and wound infection. Two animals died following caesarian section—a baboon following eight hours of anesthesia during which isotope studies of mother and fetus were performed, the other, a macaque, following an emergency section for failure to deliver spontaneously.

Table II summarizes some information on species presently available and most commonly used for experimentation. Nolan [20] has described present day methods and problems in harvesting, importing and distributing primate animals and the price list published by his firm* is the source of much valuable information.

* Primate Imports Corporation, 34 Munson Street, Port Washington, New York.

The abbreviation MQT in Table II refers to Minimum Quarantine Time. This varies among species and was established more or less arbitrarily in relation to the animals' susceptibility to tuberculosis.

Prices are quoted only in broad general terms because they vary with availability and size of the animals. All prices are F.O.B. New York, and will undoubtedly rise 10–25% during the next year.

Our laboratory (LEMSIP) has very little experience with New World monkeys (Table II). We have obtained testicular biopsies from squirrel monkeys for phylogenetic studies of spermatogenesis and we have kept a small group of capuchin monkeys being used for studies of genital herpes virus susceptibility. Marmosets are a special group of very small primates which have been very useful in studies of transplantation because of their unique production of fraternal twins in 80% of pregnancies. Husbandry has been a difficult problem, but Gengozian [21] and Deinhardt [22] have succeeded in making the marmoset a practical animal for experiments in virology, oncology, and transplantation.

The Asian monkeys (Table II) grouped in genus Macaca have been the most widely used because they are plentiful, inexpensive and readily available. The stumptail *(M. speciosa)* and pigtail *(M. nemestrina)* have recently been advocated to be good substitutes for the rhesus *(M. mulatta)* which is by far the most widely used primate. The rhesus monkey is characteristically very aggressive and carries the "monkey B virus" which is highly lethal for humans. For these reasons one must be careful to avoid direct skin contact, bites and scratches. The supply of macaques is still plentiful, and these monkeys will undoubtedly remain useful especially for experiments in reproductive physiology, surgical ablation of brain tissue, organ transplantation, etc. We have advised newcomers to the use of primate animals to consider the advantages and disadvantages carefully before choosing macaques as experimental animals, having found in our own work that baboons are often a better choice.

The African monkeys (Table II) are increasingly challenging the macaques for wide useful-

TABLE II.—*Partial Information On Species Presently Available And Most Commonly Used For Experimentation*

Animal	Habitat	Supply	Remarks
NEW WORLD MONKEYS			
Squirrel monkey *(Saimiri sciureus)*	Upper Amazon MQT—21 days	Intermittent April/August under 1 lb—$30 over 1½ lbs.—$45	Subject to transportation stress. Carries herpes saimirii virus which is carcinogenic in other species.
Capuchin *(Cebus albifrons)*	Paraquay MQT—23 days	Large animals are scarce 2—4 lbs.—$60 over 4 lbs.—$70	Susceptible to genital herpes virus
Spider monkey *(Ateles paniscus)*	Amazon Basin MQT—35 days	Scarce. On request	Phylogeny Transportation Viral studies
Marmosets *(Callithrix sp.)*	Brazil	Extremely variable some species are endangered and embargoed	Special husbandry methods
ASIAN MONKEYS			
Rhesus monkey *(Macaca mulatta)*	1) India & Pakistan 2) Formosa & Thailand MQT—42 days	Excellent up to 6 kg. about $100 Pregnant female $125 NIH certificate of need required for India monkeys	Most widely used primate Carries monkey "B" virus, and SV40 virus. Aggressive
Stumptail *(Macaca speciosa)* Pigtail macaque *(Macaca nemestrina)*	Malaya & Thailand MQT—42 days	Good, 4–8 kg. about $125	Larget than rhesus less aggressive and easier to handle
Cynomolgus macaque (crab eating monkey) *(Macaca fascicularis)*	Philippines & Malaya MQT—35 days	Good up to 3.5 kg. about $75. Pregnant female—$85	Year round supply.
AFRICAN MONKEYS			
Baboon *(Papio sp.)*	Kenya, Tanzania, Ethiopia MQT—28 days	Excellent to present Females up to 15 kg. Males up to 18 kg. about $150	Largest monkey. Hardy. No "B" virus. Excellent surgical candidate for liver, biliary tract, portal system, placentation, lung and kidney transplantation, infectious disease, reproduction.
Gelada baboon *(Theropithecus gelada)*	Ethiopia MQT—28 days	Available on request about $150	Transplantation; xenografting with baboons
African green *(Cercopithecus aethiops)*	Central & South Africa MQT—28 days	Good supply of small animals. Females up to 3.5 kg. Males up to 5 kg. about $75	Tissue culture Production of polimyelitis vaccine. *Marburg agent epidemic
Patas monkey *(Erythrocebus patas)*	West Africa MQT—35 days	Variable Females up to 7 kg. Males up to 10 kg. about $125	Infectious disease Behavior studies
ANTHROPOID APES			
Chimpanzee *(Pan troglodytes)*	West Africa	Diminishing, Potentially Endangered	Immunologically and physiologically closest to man.
	MQT—60 days	about $600/juvenile	Blood grouping, infectious diseases, (viruses. bacteria, parasites), reproduction, transplantation
Gorilla *(Gorilla gorilla)*	Central Africa	Diminishing, endangered, too difficut to handle for routine use	Phylogeny
Orangutan *(Pongo pygmaeus)*	Borneo & Sumatra	Endangered, embargoed	Phylogeny
Gibbon *(Hylobates lar sp.)*	Southeast Asia	Potentially endangered, scarce	Phylogeny and infectious disease

ness. African green monkeys *(Cercopithecus aethiops)* have been imported in large numbers to supply kidneys for tissue culture particularly for production of poliomyelitis vaccine, although this animal was the carrier of a virus which caused the "Marburg" epidemic. That event highlighted a real risk in working with primates and resulted, in Europe, in restrictions on importation of vervets. This undoubtedly contributed to the recent release of vaccines produced in diploid human cell cultures.

Baboons are becoming increasingly popular wherever surgical procedures are involved and especially in experimental surgery as applicable to man. They are large, hardy, adapt to laboratory caging and relate well to their handlers. Although large and potentially dangerous, they are less aggressive than rhesus monkeys and with proper precautions they can be used as readily as dogs. In five years of daily work with a colony averaging 150 baboons, only one technician has sustained a bite which required medical care. This record of safety is the result of individual housing in squeeze-back cages, routine use of phencyclidine hydrochloride* for tranquilization when the animals are handled, and strict discipline and careful instruction of the staff.

Baboons are intelligent and docile enough to be trained for maintenance in chaired experiments for many weeks. They may be housed easily and feeding and husbandry are not difficult.

Taxonomic classification of the baboons has been the topic of some controversy but we have chosen to follow Napier and Napier[23] in this and all other taxonomic questions. While the baboon may not be quite as "close to man" as the anthropoid apes, practical considerations of availability, price, maintenance costs and facilities make baboons the most valuable general purpose primate animal especially for experimental surgery. Its cousins, the drill, mandrill *(Mandrillus sp.)* and gelada *(Theropithicus gelada)* are too scarce ever to become regular laboratory species but the gelada is available for special studies such as xenografting of organs.

The anthropoid apes (Table II) include the genera *Pan* (chimpanzee), *Gorilla, Pongo,* (orangutan), and *Hylobates* (gibbon). Gorillas and orangs are being depleted in their native habitats and are potentially in danger of extinction. This, their large size, and difficulties in husbandry make them unsuitable for experimental purposes. However, small numbers are available in zoological collections; and in one of the primate centers, these have been used for significant phylogenetic observations.

Gibbons *(Hylobates sp.)* are found in Malayan jungles but they adapt well to laboratory conditions and have been used in studies of virus infections, gastrointestinal observations and phylogenetic studies of blood grouping and serum proteins. They are small and fragile and are not useful as experimental subjects.

Chimpanzees *(Pan troglodytes)* are potentially immensely valuable because they are, over all, immunologically and physiologically closest to man. They share human blood groups, are susceptible to human viruses and are unique among animals in their susceptibility to hepatitis virus and streptococci. Chimpanzee organs have been transplanted to human patients and have sustained human life for periods up to nine months.

Unfortunately, the growth of human populations and economy in Africa is rapidly intruding upon chimpanzee habitats and constitute a rapidly growing threat to their survival. Presently there are about 400 animals available for medical research throughout the world and one of the largest groups (71) is in the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) in New York.

The use of our chimpanzee colony for experimentation is supervised by a Utilization Committee which considers their threatened status before committing them to experimental protocols. We strongly recommend this careful regard for the future supply of chimpanzees and have been breeding our own animals as rapidly as possible. We have had three babies from four newly matured pairs during the last year. Large scale breeding of chimpanzees should be among our high priority national projects, but unfortunately, no such program has yet been funded.

* Sernylan, Bio-ceutic Laboratories, Inc., St. Joseph, Missouri, 64502.

SUMMARY

Nonhuman primate animals have been increasingly productive laboratory animal models since problems of supply, quarantine, housing, husbandry, handling, tranquilization and anesthesia have been overcome.

Surgical experience with five species of primates has been described and the present usefulness and availability of various species has been summarized.

The cost of a laboratory animal model should include considerations of "applicability to man" as well as the dollar outlay for purchase, experimentation, and maintenance. When this is done, the "cost effectiveness" of the primate model often makes it competitive with other animal models particularly for long-term studies.

As "surrogates for man" in biomedical experimentation, nonhuman primates are the most logical choices for sophisticated studies. They should be used widely with due regard for their potentially endangered status.

REFERENCES

1. Goldsmith, E. I. and Moor-Jankowski, J. Editorial. J. Med. Primat. 1:1–2, 1970.
2. Goldsmith, E. I. and Kean, B. Schistosomiasis: Experimental surgical removal of adult worms. Gastroenterology 50:805–807, 1966.
3. Hume, D. M., Gayle, W. E. and Williams, G. M. Cross-circulation of patients in hepatic coma with baboon partners having human blood. Surg., Gynec. & Obstet. 128:495–517, 1969.
4. Goldsmith, E. I. and Moor-Jankowski, J. Experimental medicine and surgery in primates. Annals of N. Y. Acad. Sciences 162:1–704, 1969.
5. Goldsmith, E. I. and Moor-Jankowski, J. *Medical primatology*, 1970. S. Karger, Basel/New York, 1971.
6. Moor-Jankowski, J. and Goldsmith, E. I. The Laboratory for Experimental Medicine and Surgery in Primates: Background and policy. In Goldsmith, E. I. and Moor-Jankowski, J. Medical Primatology 1970, S. Karger, Basel/New York, 1971.
7. Davis, J. H. and Moor-Jankowski, J. Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP): Design and operation. In Goldsmith, E. I., and Moor-Jankowski, J. Medical Primatology 1970, S. Karger, Basel/New York, 1971.
8. Muchmore, E. and Davis, J. Biological parameters of simians for medical research. Proceedings of Third International Primate Conference. S. Karger, Basel/New York, in press.
9. Moor-Jankowski, J., Muchmore, E. and Davis, J. Maintenance of a mortality free colony of primate animals for immunological, surgical, and other medical experimentation. In Balner and Beveridge: Infections and Immunosurpressions in Subhuman Primates, Munksgaard, Copenhagen, 1970.
10. Muchmore, E., Potter, J. L., Moor-Jankowski, J. and Goldsmith, E. I. Teaching program in medical primatology for medical students. In Goldsmith, E. I. and Moor-Jankowski, J. Medical Primatology 1970, S. Karger, Basel/New York, 1971.
11. Haglin, J. J. and Armer, D. Lung transplantation in the baboon. Ann. N. Y. Acad. Sci. 162:404–411, 1969.
12. Glenn, F. and McSherry, C. K. The baboon and experimental cholelithiasis. Arch. Surg. 100:105–108, 1970.
13. Child, C. G., III. The hepatic circulation and portal hyertension. W. B. Saunders Co., Philadelphia/London, 1954.
14. Ewald, B. Personal communication.
15. Goldsmith, E. I. The current role of nonhuman primates in surgical research Primates in Medicine 3:41–51, S. Karger, Basel/New York.
16. Kritchevsky, D. Experimental atherosclerosis in primates and other species. Ann. N. Y. Acad. Sci. 162:80–88, 1969.
17. Van Citters, R. L. and Franklin, D. Radio telemetry techniques for study of cardiovascular dynamics in ambulatory primates. Ann. N. Y. Acad. Sci. 162:137–155, 1969.
18. Moor-Jankowski, J. Blood groups of apes and monkeys: Human and simian types. This volume.
19. Goldsmith, E. I., Moor-Jankowski, J., Wiener, A. S., Allen, F. H., Hirsch, R. Exchange transfusion of nonhuman primates with human blood. In Goldsmith, E. I. and Moor-Jankowski, J., Medical Primatology 1970, S. Karger, Basel/New York, 1971.
20. Nolan, M. A. The logistics of the supply of primate animals for medical research. In Goldsmith, E. I. and Moor-Jankowski, J., Medical Primatology 1970, S. Karger, Basel/New York, 1971.
21. Gengozian, N. Marmosets Their potential in experimental medicine. Ann. N. Y. Acad. Sci. 162: 336–362, 1969.
22. Deinhardt, F. Current trends in use of marmosets in virological research. Ann. N. Y. Acad. Sci. 162: 551–555, 1961.
23. Napier, J. R. and Napier, P. H. *A handbook of living primates*. Academic Press, 1967.

DISCUSSION

Chairman Michael P. Kaye: Thank you very much, Dr. Goldsmith. Are there any questions on this most interesting paper?

Nicholas T. Werthessen, Brown University: I want to congratulate the speaker on his

presentation about the usefulness of these animals, but I do take issue with him on one point —his concern about the inherent danger in working with male baboons. We began to work with them in the mid-fifties, not knowing a thing about them, and had to develop handling techniques. In the set-up we had in Africa, we had separated fighting males and pushed them back into chicken wire cages. We had trouble only in one instance when an attendant deliberately teased one of the animals and was bitten. But working with several thousand of them, we never had an accident. I would much rather work with one of these animals than a stallion, a dairy bull, or even a 200 pound boar, which we consider really dangerous.

LUNG TRANSPLANTATION IN DOGS

James D. Hardy*

The incidence of chronic pulmonary insufficiency is steadily increasing in the United States. Chronic obstructive emphysema is the most prominent cause of lung disability, but tuberculosis, pneumoconioses, bronchiectasis, and chronic cystic fibrosis contribute additional cases. The work loss caused by chronic respiratory failure is substantial, and a large number of patients with such limitations eventually die from lung or secondary heart disease. Therefore, it is imperative to investigate means for relieving otherwise terminal chronic respiratory insufficiency, and the investigation of lung transplantation in animals has appeared to represent a feasible approach to the solution of this problem. While experiments have been performed in lower primates and the pig, and perhaps other animals, the dog has been most commonly utilized because of its ready availability in large numbers and the ease with which this animal can be handled. This report will deal principally with canine lung transplant research performed in our own laboratories, but related work by others will be briefly acknowledged.

INTRODUCTION

The first lung transplant recorded would appear to have been that of Guthrie in 1907, which involved the transfer of the lungs of a kitten to the neck vessels of an adult.[1] The lungs became edematous, and the experiment was terminated at 48 hours because of infection. Transplantation of the whole lung and pulmonary lobes in dogs was performed by Demikhov of the Soviet Union in 1947, but this report did not become available to the Western world until 1962.[2] Staudacher and associates[3] published results of replantation and allotransplantation of pulmonary lobes in dogs in 1950. In that same year Metras[4] reported the technique of lung transplantation which placed the anastomosis through a portion of the left atrium into which the involved pulmonary veins flowed, thus avoiding the high incidence thrombosis incurred when the pulmonary veins of dogs were anastomosed individually. This rapidly became the standard technique for present day experiments in animals. Neptune and associates[5] independently described a similar type of anastomosis one year later. Lung replantation was given strong emphasis when Juvenelle and his co-workers[6] reported the long-term survival of a dog whose right lung was replanted in 1950.

* University of Mississippi Medical Center, Department of Surgery, Jackson, Mississippi.

LUNG REPLANTATION

Lung replantation has been studied extensively in the dog, since the study of this model avoids the immunological problems which are produced with an allograft.[7–11] Thus, the physiological and other changes which occur are due to the cumulative effects of the operation of removing the lung and then replacing it in the original position, and no immunological reaction that would have been caused by transplanting genetically dissimilar tissue need by considered.

Replantation of One Lung

Operative Technique

In lung replantation the organ is removed, cooled and washed by perfusion at physiologic pressure through its pulmonary artery (Rheomacrodex or Ringer's lactate at +4°C), and then the organ is sutured back in its original position. Some workers prefer to inject heparin into the vascular tree while the organ is without perfusion with blood, to reduce the risk of small vessel thrombosis during the period of stasis. However, we have not used it routinely and we do not find it essential to do so. Furthermore, most of the blood has usually been removed from the lung by perfusion, but even this is not mandatory if the lung is promptly reinserted.

In the dog we have found the use of a left

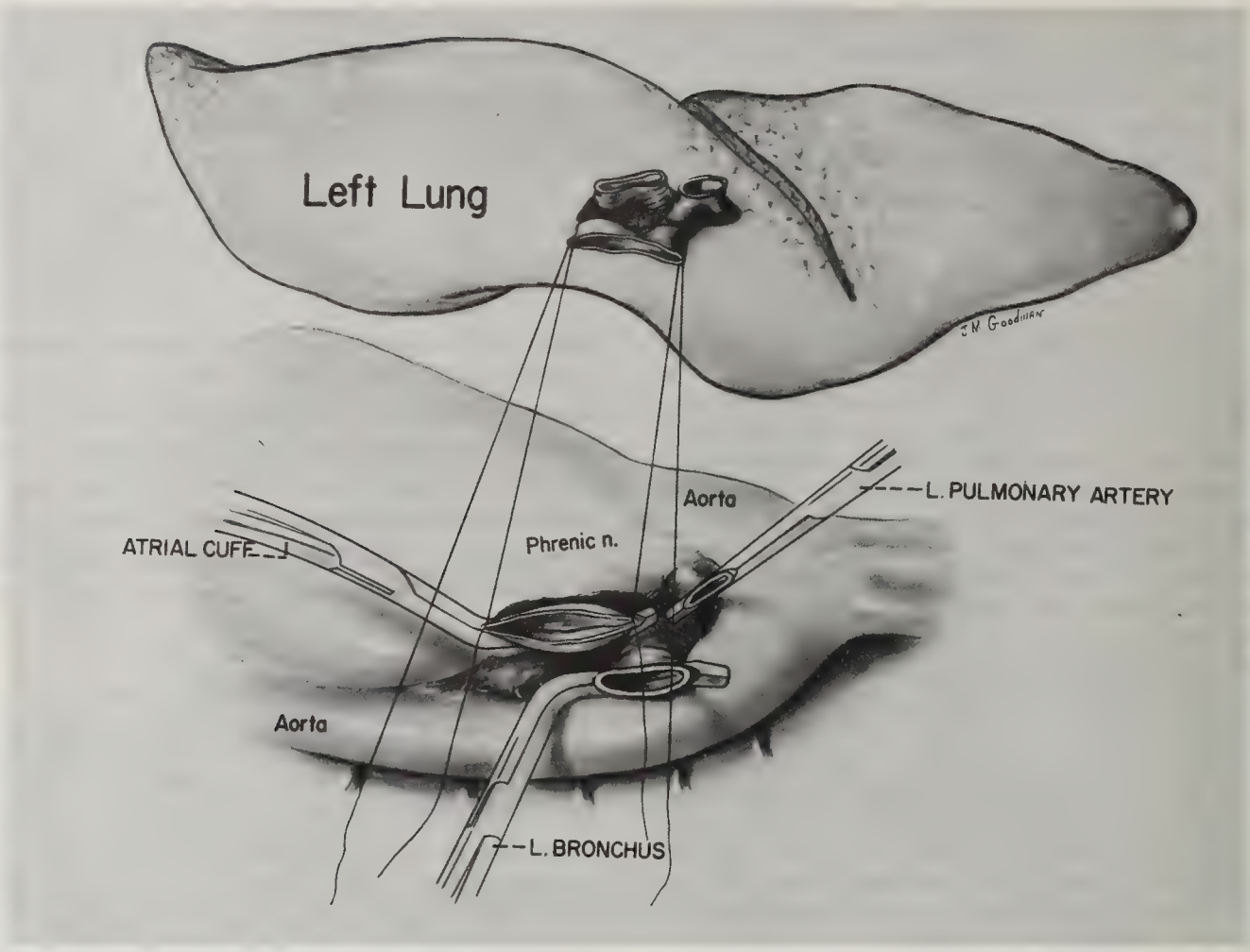
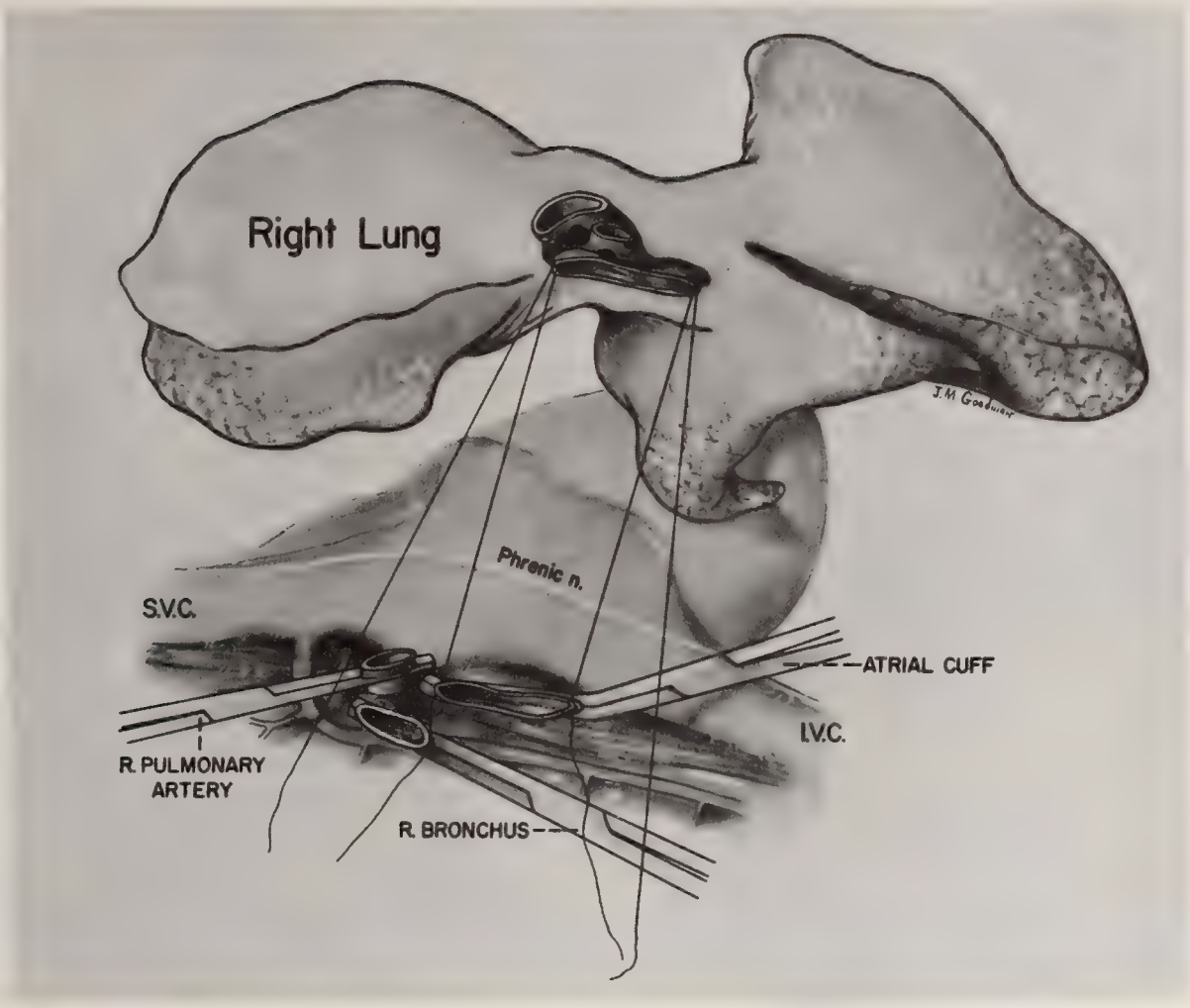


FIGURE 1.—Operative technique of lung transplantation.

atrial cuff into which flow the pulmonary veins to be the appropriate level for venous anastomosis, though anastomoses of the two pulmonary veins were used in our two human cases. In addition to the left atrial anastomosis, the operation is completed by anastomosis of the pulmonary artery and the bronchus (Figures 1 and 2). No effort is made to restore continuity of the bronchial arteries, lymphatics and nerves. A continuous suture technique is usually satisfactory, and we have commonly employed 5-0 silk. The bronchus is sutured by placing the posterior row first, achieving an everted suture line. In the experiments involving young puppies, an interrupted suture technique was used to permit growth of the anastomosed structures as the animals enlarged. Although the left lung is technically somewhat more easily transplanted, the right lung provides a greater mass of functioning lung tissue. This is an important consideration in perfecting a model where the animal must survive entirely on a replanted or allotransplanted lung. There is a certain incidence of thrombosis of the vascular structures postoperatively, but this is uncommon after the technique of lung reimplantation has been mastered.

With considerable experience in the actual operative technique and physiological management of the thoracotomy, a skillful operator can achieve successful replantation in about 75% of the animals operated upon. As noted previously, a major cause of death is stenosis or thrombosis of the pulmonary veins, or both. These complications are minimized by using an atrial cuff with careful intima to intima approximation, instead of attempting to anastomose the veins individually. An additional percentage of dogs will die from postoperative hemorrhage into the pleural cavity, pneumoni-



tis with or without empyema and, rarely, leakage from the bronchial anastomosis or thrombosis of the plumonary artery. Most deaths occur within a week, and dogs that survive the first week frequently live until sacrificed months or years later. The gross (Figure 3) and histological appearance and the angiograms of the chronically replanted lung are essentially normal.

Function of the Replanted Lung

The function of the replanted lung[12–19] is of vital concern, for it will identify the maximal functional capacity which can be expected from an allografted organ. Such information is essential to the objective of achieving successful lung allotransplantation in man. The replanted lung does function promptly but this function is rarely normal. In fact, the function of the replanted lung frequently declines to approximately one-half or less of that recorded preoperatively, after which the function of the replanted lung gradually improves fairly rapidly up until the end of the first two weeks, and subsequently slowly improves for several months. The function of the replanted lung rarely returns to the preoperative level completely, but a certain number of animals can survive on a replanted lung after either immediate or especially late ligation of the contralateral pulmonary artery. The success rate is greater when the right lung is the one replanted, since it has a greater functional capacity than the left lung.

The precise cause or causes of the reduction in function exhibited by the replanted lung have been the subject of many studies by different investigators. The cause most clearly identified is the development of pulmonary edema in the replanted lung, though the precise explanation of this edema seen in roentgenograms is not certain. It is clear that the lung is definitely damaged by periods of ischemia, despite the fact that the lung tissue can actually

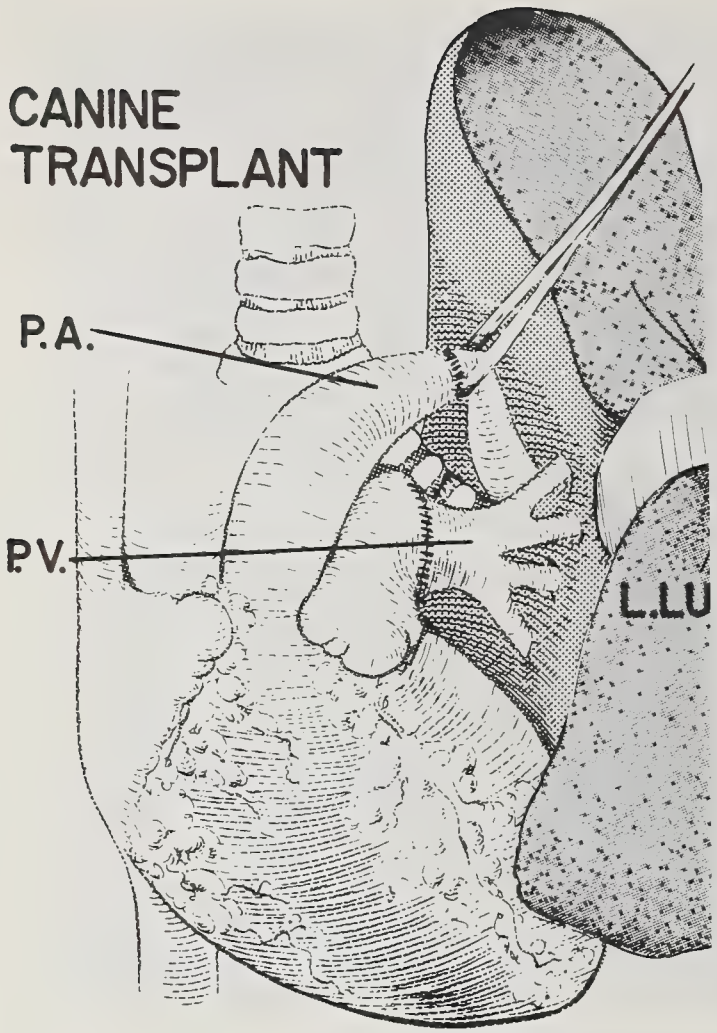


FIGURE 2.—Transplantation of left lung. It is essential to avoid constriction of pulmonary veins, pulmonary artery or bronchus.

survive two hours of cross-clamping of the entire hilum.[20] Therefore, the lung should be cooled by perfusing the pulmonary artery with cold solution at a low pressure, followed by expeditious and very gentle replantation. Trauma to the lung or liver during the course of replantation can produce damage which will be reflected in reduced function. Inevitably some degree of atelectasis and pneumonitis develop.

For a time it was believed that division of the pulmonary lymphatics was a cause of the edema and diminished function which the replanted lung exhibits, and certainly the late postoperative improvement in function of the replanted lung parallels the reestablishment of lymphatic channels across the bronchial suture line. Nevertheless, the fact that the curves representing diminished lung function and diminished lymphatic flow parallel each other does not necessarily prove that the diminished lung function is due to lymphatic stasis during the period prior to regeneration of the lymphatic channels. Other workers have concluded that the division of the hilar elements, especially the nerves to the lungs, results in alterations in the pulmonary surfactant, but different investigative teams have reported widely varying results in this regard.[18,19,21] Ciliary action and the clearing of bronchial secretions is somewhat impaired in the replanted lung,[22,23] but ciliary action is not completely abolished by dividing all elements of the pulmonary hilum. Still other groups have called attention to the fact that the bronchial arteries are divided with lung replantation and that usually no attempt is made to restore continuity of these vessels.[24] In the late post-replant period the bronchial arteries do reestablish anastomotic connections with the pulmonary artery and the pulmonary veins, in the bronchial mucosal plexuses.[25]

The capacity to transmit oxygen to the arterial blood is relatively more impaired in the replanted lung than is ventilation. The most valid studies are achieved by blocking the opposite pulmonary artery with a ballon-tipped catheter or by ligation of this artery. Of course, contralateral pneumonectomy absolutely establishes the quality of function of the replanted lung. In addition to these and still other measurements of lung function, the use of radioactive xenon (Xenon–133) will give information regarding both the distribution of lung perfusion and the distribution of lung ventilation, depending upon whether the material is injected intravenously or is inhaled with the gas mixture.

In summary, temporary reduction in the function of the replanted lung is usually due to a number of factors, some of which have clearly not yet been identified. Furthermore, the relative importance of individual factors which have been identified probably varies from animal to animal, and certainly they vary in the opinion of different groups of workers from different laboratories. However, a meticulous transplant technique permits the best possible post-transplant function.

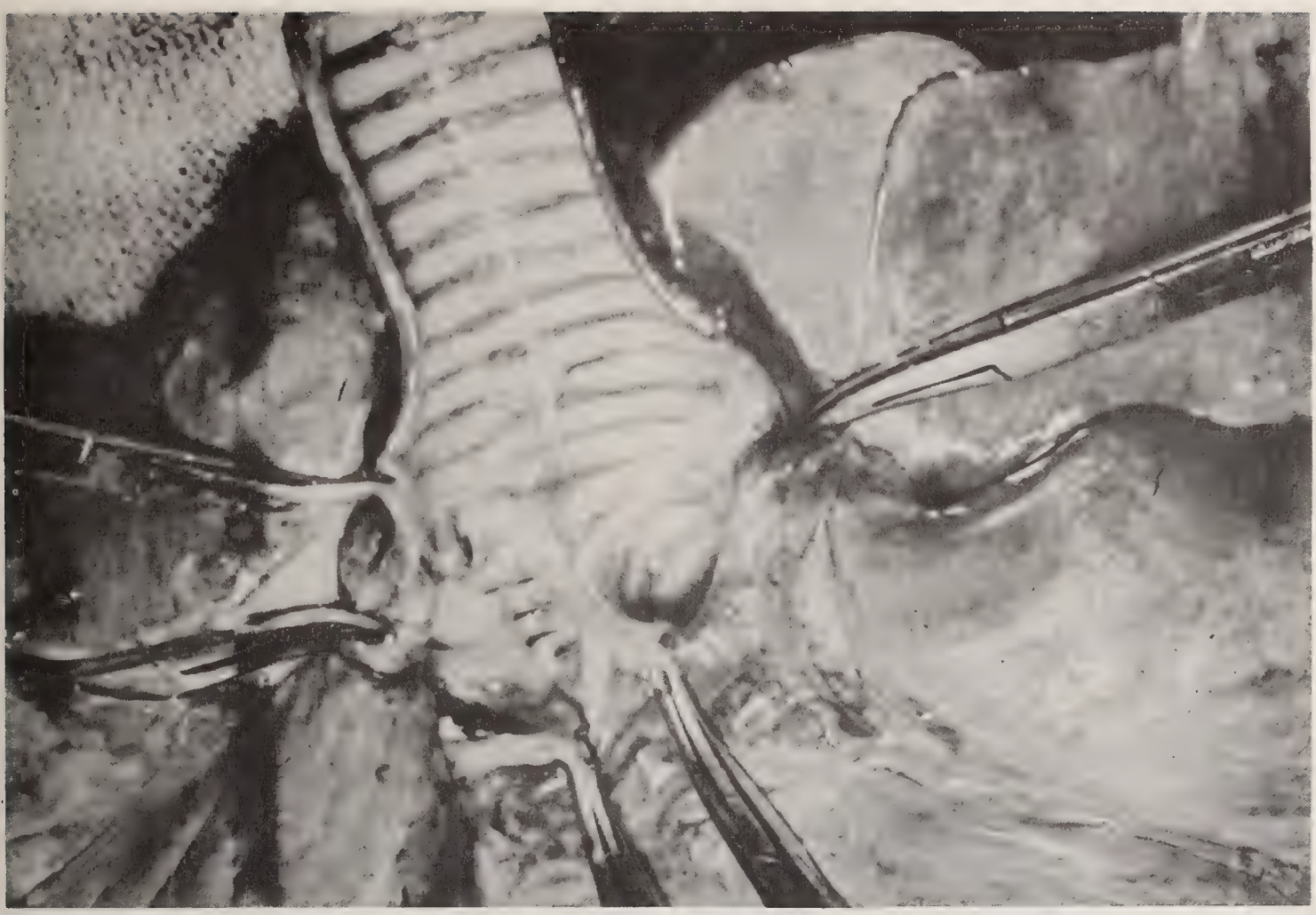


FIGURE 3.—Bronchial suture line months following left lung replantation. There is no stenosis.

Complications of the Bronchial Anastomosis

Brief mention has been made of the hazard of vascular stenosis and thrombosis. Thrombosis of the pulmonary artery is uncommon and occlusion of the pulmonary veins is relatively infrequent in the hands of experienced operators using the left atrial cuff technique.

Bronchial complications will occur in a certain number of animal experiments, and this problem has also been reported in several human cases. Leakage at the bronchial anastomosis may result in infection, hemorrhage or pneumothorax. The bronchus and its mucosa are able to derive sufficient blood supply from the pulmonary artery, through a network of interconnecting small vessels in the bronchial mucosa. Thus, when the bronchial artery pressure is lowered by ligation of the bronchial arteries, pulmonary artery blood enters the mutual capillary bed to supply the bronchial mucosa, though with a less oxygenated blood than is normally the case.

The integrity of the bronchial artery does not appear to be important for the blood supply of the main stem bronchus from the carina to the origin of the upper lobe bronchus in man. Accordingly, it would appear advisable to leave the main stem bronchus of the recipient lung long, and to divide the bronchus of the donor lung fairly close to the origin of the upper lobe bronchus. Furthermore, excessive stripping or denuding of the bronchus of either donor or recipient should be avoided. Finally, we prefer to cover the bronchus with surrounding tissue when such tissue is available, though frequently it is not.

Post-transplant bronchoscopy will frequently reveal a loss of bronchial mucosa at the suture line, but fortunately most animals do not exhibit

clinically detectable leaks at the bronchial anastomosis. Late bronchial stricture develops occasionally.

Regeneration of Lymphatic Channels in the Replanted and the Allotransplanted Lung

As part of a systematic investigation of the causes of post-replantation pulmonary edema and diminished lung function, we investigated the rate of regeneration of the lung lymphatic channels in 15 adult mongrel dogs.[26] The 12 survivors were variously evaluated at 24 and 48 hours, 3, 7, 12 and 20 days, 6 and 7 weeks, and 1 year after replantation. In brief, Sky-Blue Dye (1 to 2 cc's) was injected into the replanted lung tissue by the technique described by Weinberg. Spreading of the dye in the lung tissue was noted, and 25 to 45 minutes after its injection the animal was sacrified. The lungs were removed, carefully examined and photographed. Attempts to demonstrate the regenerating lymphatics with radiopaque materials failed because of the small size of these lymphatic channels. However, when the blue dye was injected into the replanted lung 24 hours after replantation, it spread towards the hilum and the peribronchial lymphatics within 45 minutes. The divided large and small lymphatic channels were clearly visible around the left main stem bronchus but did not cross the suture line (Figure 4). No dye was found in the peribronchial lymph nodes at this time. Likewise, even on the third day there was no evidence of migration of the dye across the suture line. The left main bronchus distal to the anastomosis did exhibit a blue color up to the suture line, but the proximal portion and the trachea remained unstained. In contrast, the dye injected into the lung of the dog re-operated upon one week later spread to the hilum. Although gross-

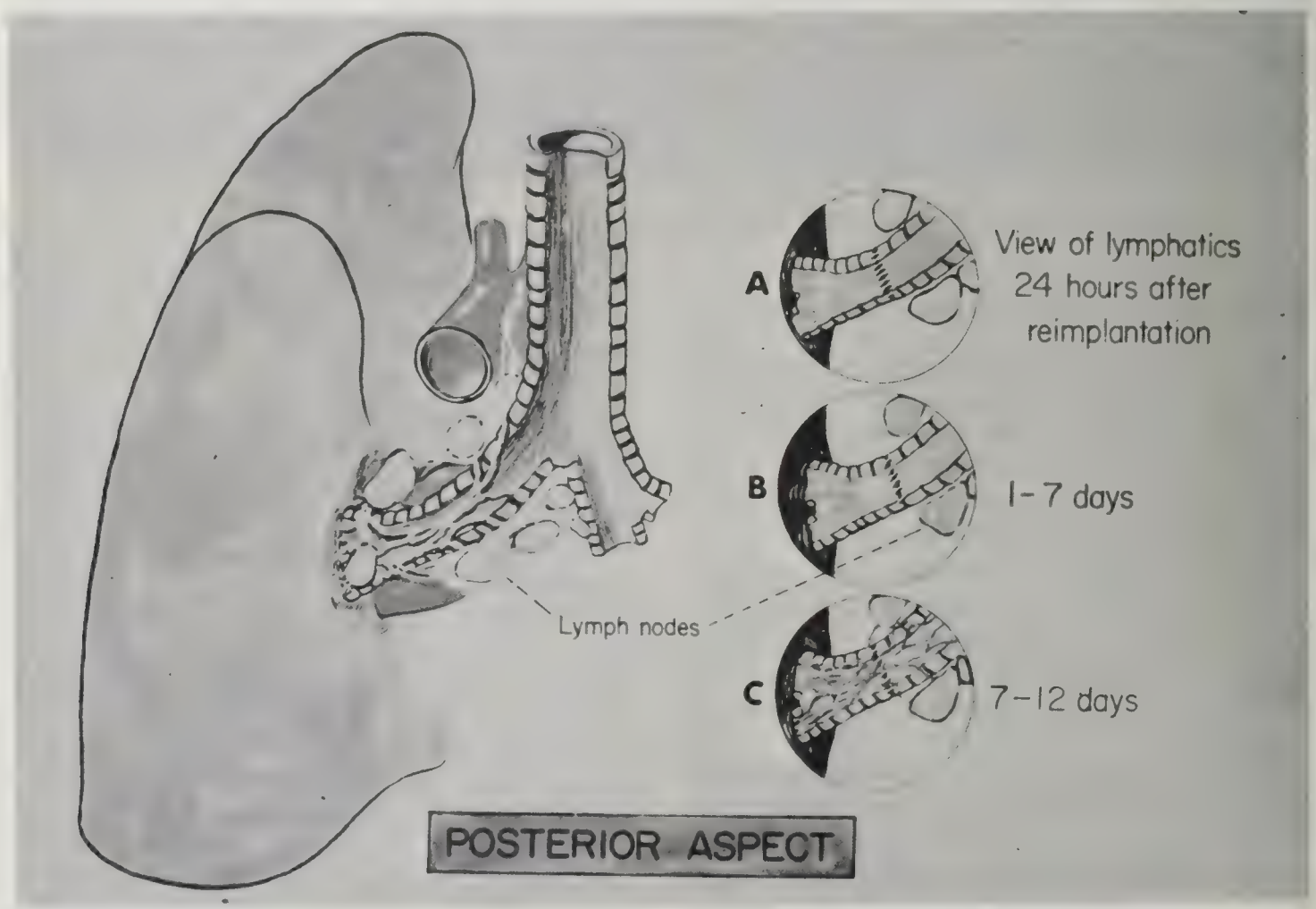


FIGURE 4.—Regeneration of lymphatics occurs readily in both replanted and allotransplanted lungs.

ly there were no visible lymphatic vessels, it was evident that the adjacent pair of hilar lymph nodes were stained within 45 minutes. By the 12th postoperative day not only were the adjacent lymph nodes stained, but also a few small lymphatic vessels were visible running across the bronchial anastomosis. By the 20th day, many stained lymphatic vessels could be demonstrated crossing the suture line and entering the peribronchial lymph nodes, and some of these channels coursed directly to the trachea. Furthermore, the newly regenerated vessels were almost as large as those seen in the normal lung. Incidentally, we found that the lymphatic channels were restored as readily in the allotransplanted lung as in the replanted lung.

At that particular time in the course of our long-term investigation of lung transplantation, we concluded that the regeneration of lymphatics might play a significant role in the dissipation of the pulmonary congestion which usually followed replantation of the lung. The regeneration of lung lymphatics had of course been studied by others in the past.

Nerve Regeneration Following Lung Replantation

It was long believed that the innervation of the major bronchi was essential to provide adequate respiratory reflex drive, and thus the capacity of pulmonary nerves to regenerate was accorded considerable investigation in the early years of lung replantation studies.[27–30] Parenthetically, it may be noted here that it has been shown by simultaneous bilateral lung replantation that the pulmonary nerves are not essential for adequate respiratory function, though it would be misleading to imply that pulmonary innervation is not important in achieving normal efficiency of respiration.

The Hering-Brener stretch reflex is absent in the first weeks following lung replantation (Figure 5). In general, it appears that there is early degenerative change in the vagus nerve fibers following replantation, as seen in serial sections taken at varying number of days (Figure 6). However, re-examination of the lung with biopsies taken a number of months later shows stability of the nerve, with normal-appearing unmyelinated nerve fibers (Figure 6). Thus, there is partial anatomic recovery of the vagus nerve, but the degree and frequency of return of anatomic and functional integrity of the vagus nerve months or years after replantation is still a controversial point. Marshall and Gunning[23] reported the return of the Hering-Breuer stretch reflex in a dog almost 4 years after replantation of the left lung. In one animal so studied it appeared that ciliary activity, as reflected in the upward progress of a small amount of India ink, was essentially normal. (It should be noted here that ciliary activity is not completely dependent upon the integrity of the nerve supply to the lung.) In contrast, in a second animal the rate of ciliary movement on the replanted left side was only about one-third of that on the normal right side. These workers found no evidence that the function of the lung had deteriorated as long as 4 years after replantation. In sum, it is now accepted that the nerves to the lungs do regenerate in most animals, in varying degrees. Innervation is not essential for adequate respiratory function to permit survival, but the pulmonary innervation does contribute to the overall quality and efficiency of respiration.

Differential Division of Hilar Tissue

A study was undertaken to identify the effects of differential division of the bronchial arteries,

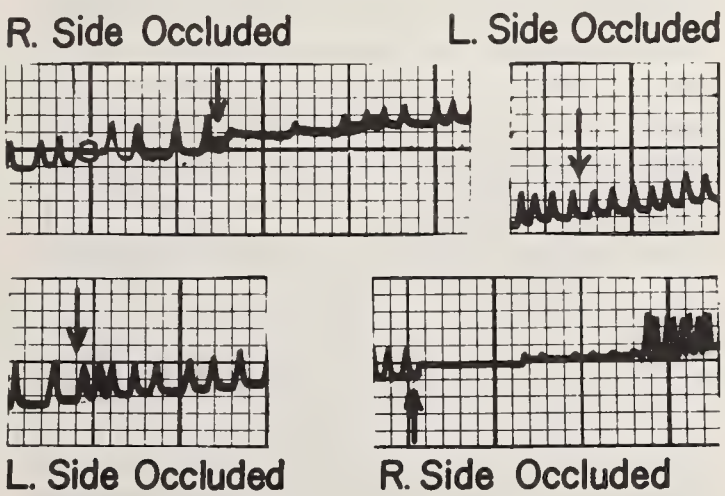


FIGURE 5.—Effects of nerve division. Note that occlusion of the main bronchus to the replanted left lung, producing lung distention, caused no change in respiratory rhythm. However, distention of the innervated (normal) right lung promptly produced apnea.

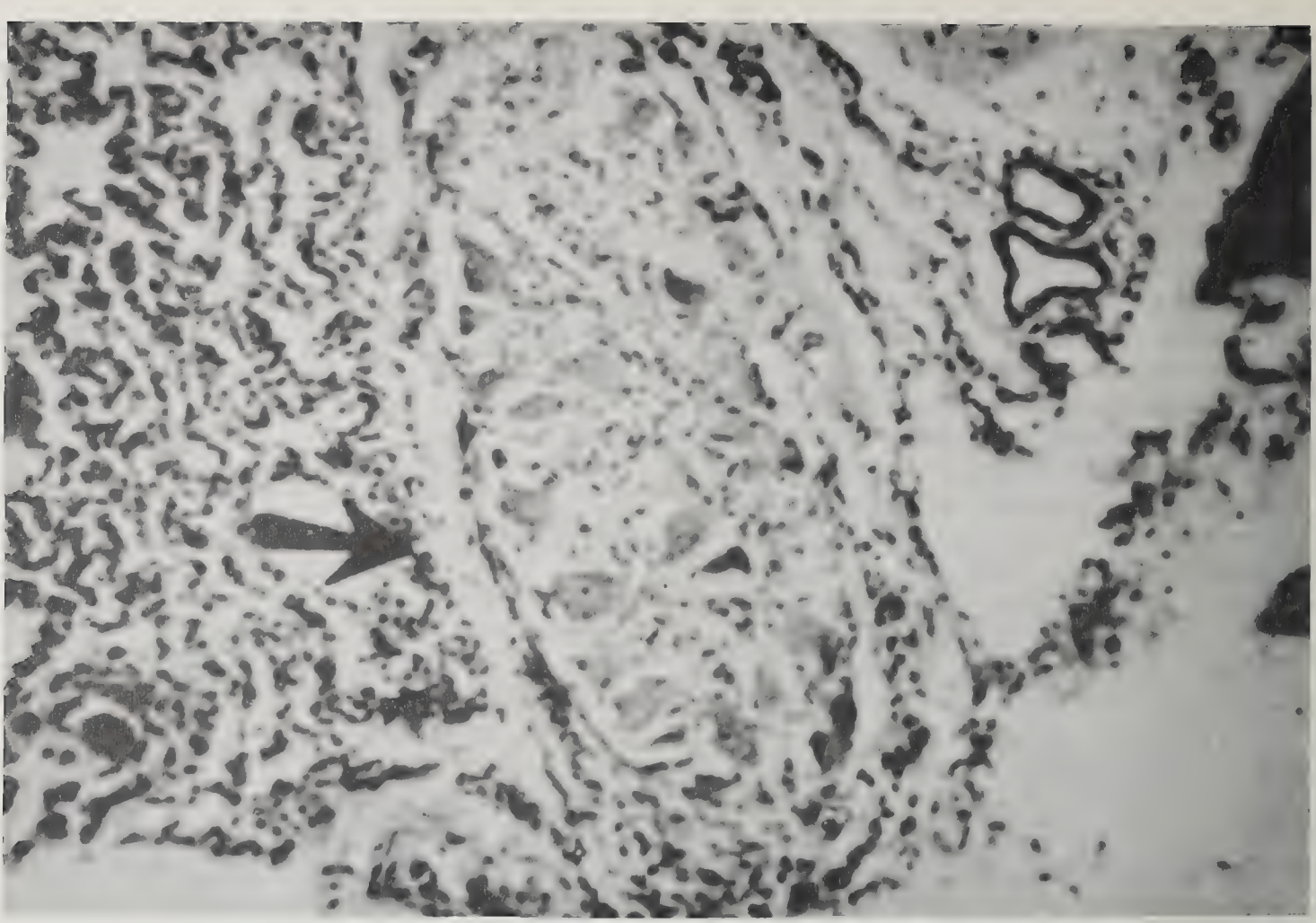
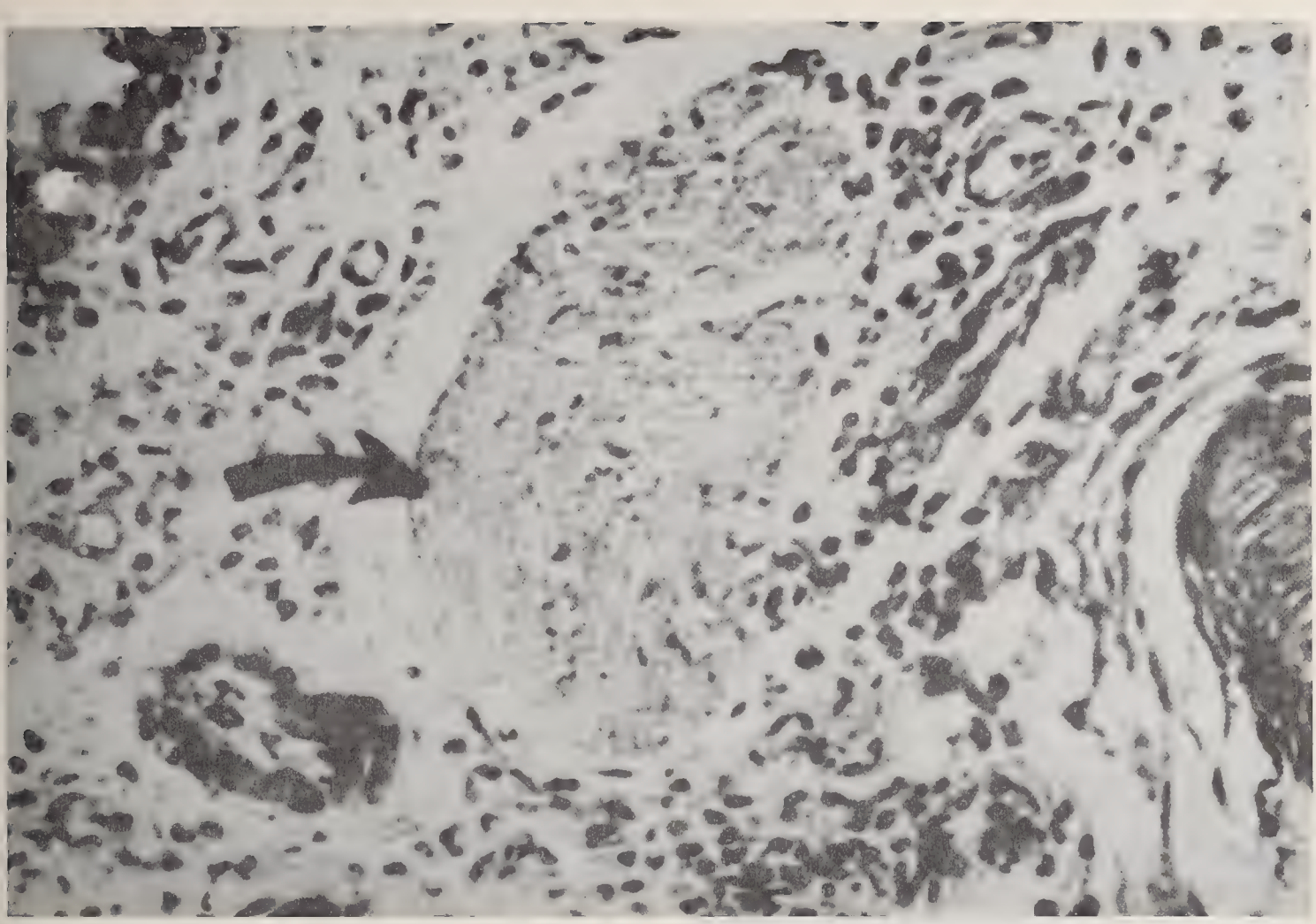


FIGURE 6.—Above. Degenerative changes in pulmonary nerve early following lung replantation. Facing page. Stable microscopic appearance of nerve two years after lung replantation.

lymphatics, the vagus nerve and a combination of these without actual division of the pulmonary artery, the pulmonary veins, or the main stem bronchus (Figure 7). Fifty-four adult mongrel dogs were divided into eight groups of experimental and control animals.[31] The results indicated that the division of all hilar tissue—including nerves, lymphatics, bronchial arteries and hilar adventitial arteries —usually caused a significant decrease in oxygen uptake, which was also seen after denervation only. Edema often developed on the operated side, which undoubtedly reduced pulmonary function following hilar stripping. While the effect was not great, the loss of vagal innervation was found to impair capacity for both ventilation and oxygen uptake. Division of the bronchial arteries and the lymphatics had significant effect upon lung function. Thus, while it had been shown in other experiments that an intact pulmonary nerve supply was not absolutely essential for survival of the animal, there appeared to be little question that denervation did impair the functional capacity of the lung to a degree.

Anatomical and Functional Growth of the Replanted Lung in Puppies

Over the years a number of children with cystic fibrosis and severe pulmonary disability have been presented to us for possible lung transplantation, but these subjects with chronic pulmonary infection have not appeared to be suitable candidates for this procedure at the present stage of its development. Nevertheless, it seemed important to determine in the animal model whether or not a lung replanted in the



young would grow both in size and in functional capacity with the growth of an animal as a whole.

We investigated the growth of lungs replanted in puppies (Figure 8). After a considerable number of unsuccessful attempts we achieved four living animals.[32] These dogs were studied one year following a left lung replantation, at which time their body weights had almost doubled since the date of lung replantation. Endoscopy disclosed that the bronchus was narrowed approximately 20% in three puppies but was narrowed almost 50% in the fourth puppy. Arteriography demonstrated an absence of either arterial or venous stenosis and showed a normal lung vascular pattern. In the one dog sacrificed for histological studies at that time, the replanted lung was grossly and microcopically normal. The tidal volume and the minute volume of the replanted left lungs varied from 46 to 50% of the total in three puppies which did not have bronchial stenosis.

Thus, it was clear that the lung replanted in a puppy did have growth potential with respect to both anatomic size and functional capacity. These findings have since been confirmed by others.[33]

Lung Storage

In preparation for possible clinical application of lung allotransplantation, we investigated various parameters of lung storage for periods of up to 48 hours in 101 dogs.[34] The excised lung was perfused briefly with cold solution and placed in a refrigerator at 4°C. It was found that the lung withstood ischemia for three hours (2 hours of storage plus 1 hour for the replantation procedure) with a functional impairment that was only slightly greater than that which followed immediate transplantation of the lung. However, storage under these conditions for longer periods of time resulted in rapidly decreasing func-

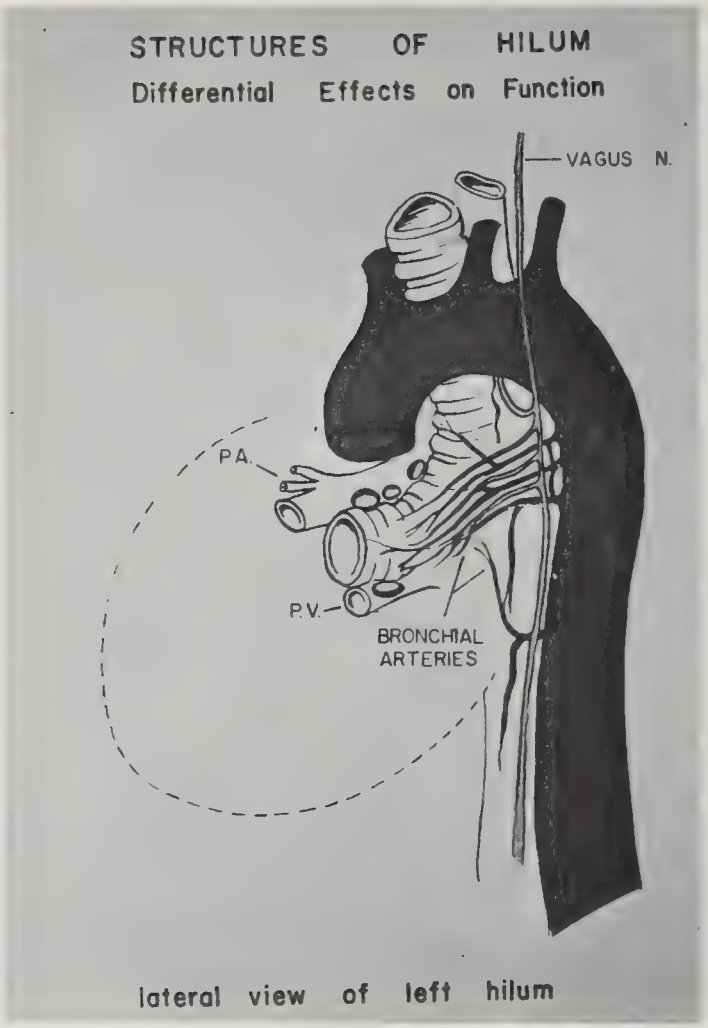


FIGURE 7.—The various structures of the pulmonary hilum were divided individually to determine effects on lung function. Only nerve division appeared to have a significant deleterious effect.

tional capacity. Even so, lungs stored for 24 hours under hyperbaric oxygenation at 43°C and then replanted were able in some dogs to provide a normal Po_2 and Pco_2 in arterial blood when the animals were subjected to contralateral pneumonectomy 6 months later. Nevertheless, these animals were unable to survive more than a few hours on the replanted stored lung due apparently to increased pulmonary arterial pressure and right failure. Cell viability studies with the trypan blue technique indicated a significant level of cell survival after even 72 hours of lung storage under hyperbaric oxygenation at 4°C.[35] Yet, it should be noted here that disagreement exists regarding the value of hyperbaria, and extended lung preservation through continuous perfusion of the pulmonary artery should be further investigated.

It was concluded from the above studies and those reported in the literature by others that a functioning human lung allotransplant could be readily achieved if certain conditions were observed. First, the blood probably should be flushed from the organ by brief perusion of the pulmonary artery with saline or a colloid solution (Rheomacrodex) containing penicillin and heparin, perhaps with added xylocaine. Second, the perfusion solution should be cold, to reduce the temperature of the lung promptly, in order to diminish metabolic requirements for oxygen and hopefully to reduce the injury caused by ischemia and hypoxia. Third, in certain circumstances intermittent ventilation of the bronchus with 100% oxygen should be useful during operation in achieving some oxygenation of the lung while the various structures were being anastomosed in transplantation.

It was thus established that a functioning lung replant or allotransplant could be readily achieved if the period of hypothermic hypoxia was not prolonged for more than 2-3 hours. Much research has been and is still being conducted for the development of better methods of organ storage in general, and these include studies of lung preservation as well.[36–39]

Pulmonary Lobe Replants and Lung Replants in Other Species

Adams and his associates[40] have studied lobar transplantation extensively in the dog. By and large, the information obtained has not varied significantly from that achieved through replantation of the lung and, from a practical point of view, we have not considered lobar transplantation to offer a sufficient advantage to accept as a model, as compared with replantation of an entire lung.

While our assignment in this symposium is that of lung transplantation in dogs, it should be noted here that outstanding work has also been carried out with baboons. Haglin and associates[41–43] found that the baboon could readily survive on a single replanted lung, whereas dogs were rarely able to do so. However, in recent years the impoved operative technique and postoperative management em-



FIGURE 8.—The lung replanted in puppies grew in both size and functional capacity.

ployed in lung transplantation in dogs has often achieved survival of animals on only the replanted lung, when the pulmonary artery was ligated immediately or some months after the replantation. Again, the success rate is greater when the right lung is replanted, due to the larger functioning lung tissue mass represented by the right lung as compared to the left lung.

Left Lung Replant With Immediate Ligation of the Right Pulmonary Artery

In preparation for study of the immediate bilateral lung replantation model, we performed left lung replantation with immediate ligation of the right pulmonary artery through the same left thoracotomy incision.[44] A number of dogs survived (Figures 9 and 10). Hill and Shaw achieved long-term survival in dogs after pulmonary replantation and staged contralateral pneumonectomy.[45]

Bilateral Lung Replantation

Thus far the discussion has been limited to studies performed by us and others using the model of a single replanted lung, since for a number of years it was believed that the neural connections to at least one lung were essential to provide adequate reflex respiratory drive to permit survival of the animal. This concept began to be challenged in the early 1960's, when occasionally various groups of workers were able to achieve survival of an animal in which both lungs had been replanted.[46,47] It was perhaps first well demonstrated by Faber and his associates[48] in a series of dogs in 1965 that if the right lung were replanted first, the dog could survive replantation of the left lung approximately one week later in some instances. Still

later, it was further demonstrated,[49] and particularly by Alican and associates,[50–52] that with exquisite operative technique and meticulous postoperative management a substantial percentage of dogs could be made to survive bilateral replantation of the lung performed at the same operation. Such success by several groups of investigators represented a major breakthrough in the development of lung replantation as a model for the study of numerous parameters of lung function.

In the early months of 1970, we performed simultaneous bilateral lung replantation in 26 dogs. A light anesthesia consisted of intermittent intravenous doses of thiamylal (Surital) sodium, and a respirator delivering a 40-60% oxygen-air mixture provided control of respiration. In each dog, first the right lung was removed and replaced through a thoracotomy in the right fifth intercostal space; then the animal was turned to the other side and left lung was removed and replaced through a similar incision on that side. The excised lung was cooled by perfusion through its artery with 500 cc's of lactated Ringer's solution at 4°C at a pressure of 60 centimeters. Ten dogs lived more than one month, and seven of these lived indefinitely (Figure 11). Most of the remaining 16 dogs died during the first postoperative week, the principal cause of death being severe pulmonary edema. Various degrees of pulmonary edema were also observed in the animals that eventually became long-term survivors. As a rule, the x-ray films of the chest were remarkably clear on the first postoperative day. Diffuse edema in both lung fields often developed by the third day, but it began to subside during the second week and disappeared during the third week of survival (Figure 12). In chronic survivors, the lung scans (Figure 13) and pulmonary angiograms (Figure 12) were normal, where performed. Pulmonary arterial pressures were found to be normal in 5 and moderately elevated in one of the long-term survivors. Blood gas values were also within normal limits.

Most of the research in the lung replantation field has been largely with the animal model employing the replantation of only one lung. Of course, since the animal can readily survive on the other lung, the technical imperfections and the actual amount of lung function contributed by the replanted lung have often been difficult to assess. In marked contrast, the functional capacity of the replanted lungs in the bilateral replantation model is beyond question. Our experiments with bilateral lung replantation were performed after the bilateral replantation technique had been thoroughly standardized in our laboratory. The fact that 38% of the dogs subjected to a one-stage operation of this magnitude lived more than one month had an important bearing on the possible application of simultaneous bilateral lung replantation in human patients with severe pulmonary emphysema. The success of these experiments also laid to rest the previous uncentainty regarding whether or not the animal with simultaneous lung replantation would have sufficient respiratory reflex drive to maintain adequate respiration. The principal cause of early death in our bilateral simultaneous replantation experiments was pulmonary edema which occurred without evidence of thrombosis or narrowing at the atrial cuff suture lines. The course of this edema was followed by means of x-ray films of the chest and clinical findings as well. The pulmonary edema was generally manifest on x-ray films after the first postoperative day, and its progression was dependent upon the degree of activity (and consequent pulmonary blood flow) of the animal. Dogs which were not confined to their cage during the first few days sometimes rapidly developed pulmonary edema. For example, three dogs which were permitted to walk to the x-ray department during this period succumbed to pulmonary edema in a matter of hours after they had struggled vigorously against restraint during positioning for x-ray films. It appeared that the edema was largely due to anoxic and mechanical injury to the microvasculature of the graft during the operative procedure of replantation. However, this injury proved to be potentially reversible. The tendency to edema seemed to be intensified by any increase in cardiac output in the postoperative period. It appeared possible that interruption of the lymphatic vessels of the lung might have played a part in its development, since the subsidence and disappearance of the



lung replantation and immediate right pulmonary ligation.

edema within the first weeks coincided with the usual time of lymphatic regeneration. Subnormal Po_2 values were frequently noted in the first two weeks, but these values returned to normal with the disappearance of pulmonary edema on chest roentgenogram.

Workers in the field of lung transplantation had commonly believed that the canine lung would readily tolerate several hours of ischemia when cold. However, this view had been based on unilateral (or staged bilateral) replantation experiments in which the reversible but significant damage that the graft suffers during hypoxic transfer was not necessarily detrimental to the animal, since the other lung remained intact. In contrast, when the life of the animal was dependent solely on replanted pulmonary tissue, as in our current experiments, it became clear that effective preservation of the graft while it was anoxic was the most crucial step in the technique of one-stage replantation of both lungs. The quality of the function of the replanted lung depended upon the rapidity and uniformity with which it was cooled. The most rapid and uniform cooling of the lungs was achieved by the infusion of lactated Ringer's solution through the pulmonary artery, in contrast to earlier usage of the colloid dextran. Incidentally, it was our conclusion that the relatively poor function of the second lung transplant which we performed in man was due to



FIGURE 10.—Lung scan in Dog #11 (see Figure 9) six weeks after operation.



FIGURE 11.—Colony of dogs late following bilateral lung replantation at the same operation.

the relatively prolonged warm ischemia time of the donor organ in that patient, as contrasted to the better function of the first clinical lung transplant that we had performed.

With respect to bilateral pulmonary denervation and respiratory reflex drive, it was evident to us that dogs which underwent simultaneous bilateral lung replantation were at a definite disadvantage with regard to ventilatory adjustments, this disadvantage lasting from several hours to two days following the operation. Pulmonary blood flow also is influenced by pulmonary innervation.[8]

This excellent model of bilateral simultaneous one-stage lung replantation has suggested that bilateral simultaneous lung transplantation should be technically and physiologically feasible in man, and indeed this operation has already been performed in human beings several times in other centers.

LUNG ALLOTRANSPLANTATION

Allotransplantation of the lung has been extensively studied in dogs [53-57] and has also been performed in 28 human beings. The operative technique employed for lung allotransplantation in the dog is essentially identical to that used for lung replantation, except that the lung transplant is derived from a second and genetically dissimilar animal. In fact, allotransplantation of the lung is more easily accomplished than replantation because with the former the structures to be anastomosed can be conveniently left long—this when one dog was utilized merely as the donor and the other as the recipient, as is usually done. However, with careful technique and using two teams, one can usually exchange lungs between dogs, thus achieving two experimental animals at the same operation. More recently, we have employed the



FIGURE 12.—The 12-a, 12-b and 12-c photographs demonstrate rather typical serial chest roentgenograms following bilateral simultaneous lung replantation. Note maximal changes at 3 days, followed by progressive clearing. Figure 12-d presents a bilateral pulmonary arteriogram 4 months following bilateral lung replantation at the same operation.

simultaneous bilateral lung allotransplantation model in dogs, to force the animal to survive completely on allotransplanted tissue. This completely removes the nagging question of whether or not the allotransplanted tissue is furnishing significant respiratory support when one of the dog's own lungs remains in place.

In the dog, the lung allograft is usually rejected in approximately 5–7 days, if measures are not employed to minimize or suppress the rejection reaction (Figure 14). Actually, the reaction is so severe that the recipient usually dies in a few days. However, occasionally a dog will survive necrosis of the transplanted lung, this being due to the fact that the organ becomes encased in a fibrous capsule and thus is sealed off from the general metabolic pool.

Perhaps the earliest experiments at prolongation of lung allograft survival were those of Hardin [58,59] and Neptune [60] and their associates. Hardin's group found that a control group of lung homograft recipients survived for 12 days and that microscopic examination of the lung allotransplants suggested that the changes were due to tissue incompatibility. They achieved longer survival when the donors and recipients were litter mates. Cortisone therapy also appeared to increase the length of survival time. Neptune and co-workers performed 25 homologous lung transplants and described the details of the usual clinical course and pathology. The average survival time without the use of ACTH was approximately 6 days, whereas ACTH injection in 4 animals resulted in an average survival time of 25 days. Blumenstock and as-

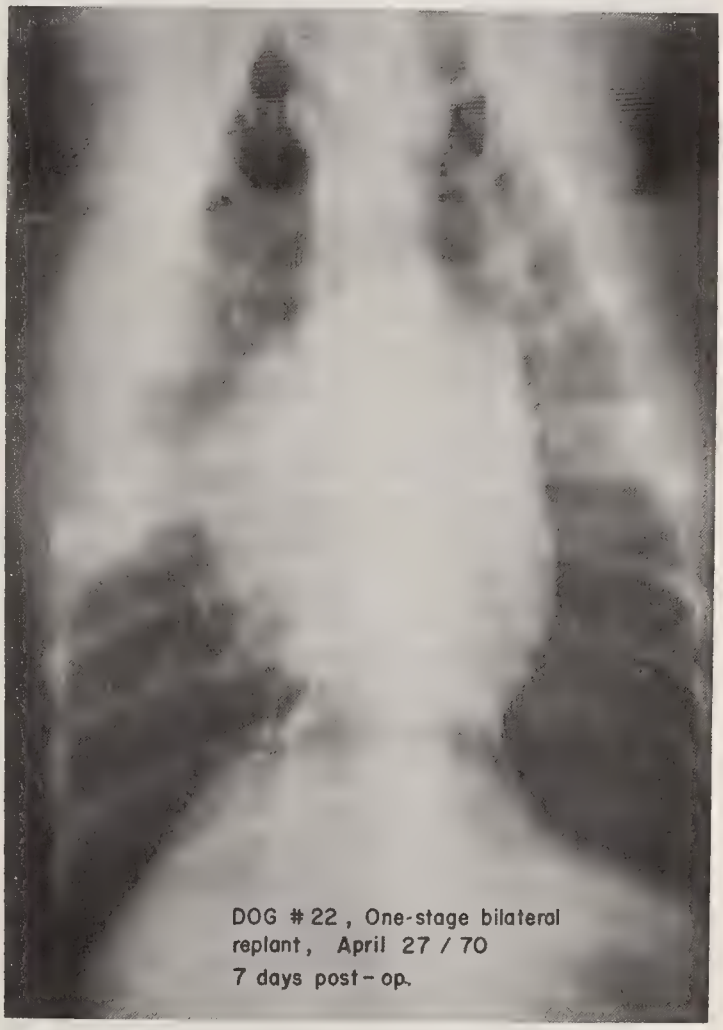


FIGURE 12b.

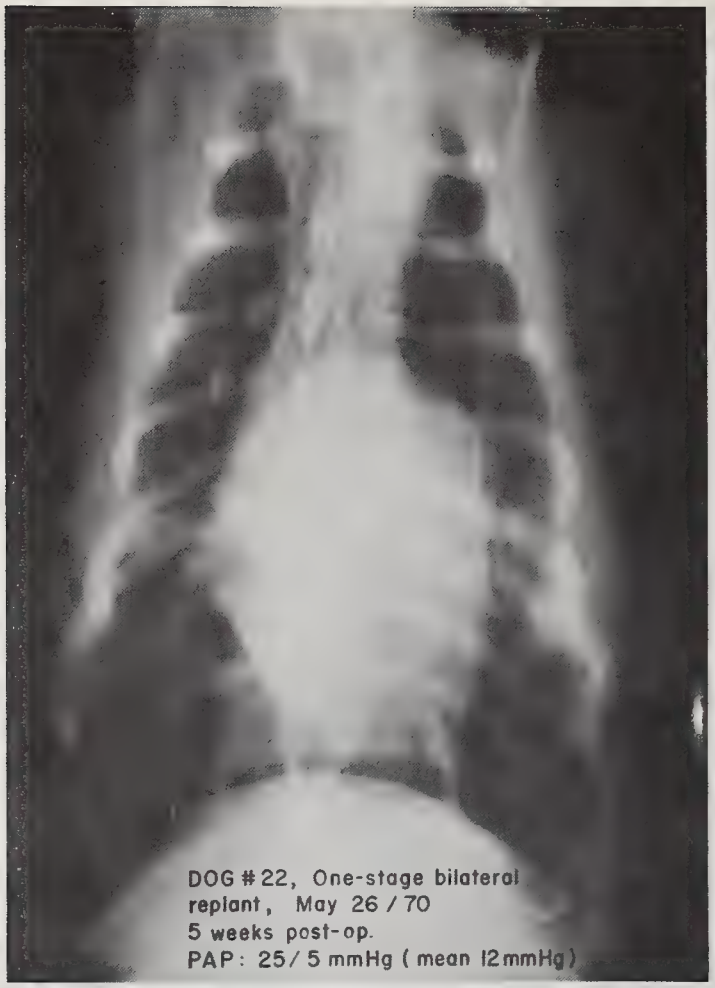


FIGURE 12c.

sociates [61-64] studied rejection extensively, with and without methotrexate suppression. Animals exhibited evidence of a progressively more severe reaction after the second day, characterized by listlessness, cough, and a thin foamy sputum. These workers were later to describe long-term survival in several animals using methotrexate as the immunosuppressive agent. The histologic features of lung allograft rejection have been well described in the literature and certain of these features are presented in Figure 14. Vascular changes are important.[65,66]

Our initial studies of lung allotransplantation were performed in a series of 138 dogs with the particular objective of determining the efficacy of various means of immunosuppression.[67] The treated groups all lived longer than did the controls, the control animals living an average of 7.4 days. Twenty of 34 animals treated with azathioprine (Imuran) in a dosage of 4.0 mg per kg per day by mouth survived the immediate postoperative period and lived an average of 30.4 days. Incidentally, the angiograms in one animal which was a permanent survivor were remarkably normal on the day prior to sacrifice, despite the fact that the lung was found to have been encased in a fibrous envelope and essentially rejected. This again emphasized that only by differential lung function studies, or by ligation of the contralateral pulmonary artery or by contralateral pneumonectomy, could one be certain of the functional status of the allografted lung when one of the animal's own lungs remained in situ.

Various other immunosuppressive regimens have been employed in our laboratory, including radiation to the mediastinum, horse anti-dog lymphocyte serum (or globulin) and prednisone. The best average survival rate that we have achieved in any series has been 35 days, this being accomplished with a combination of

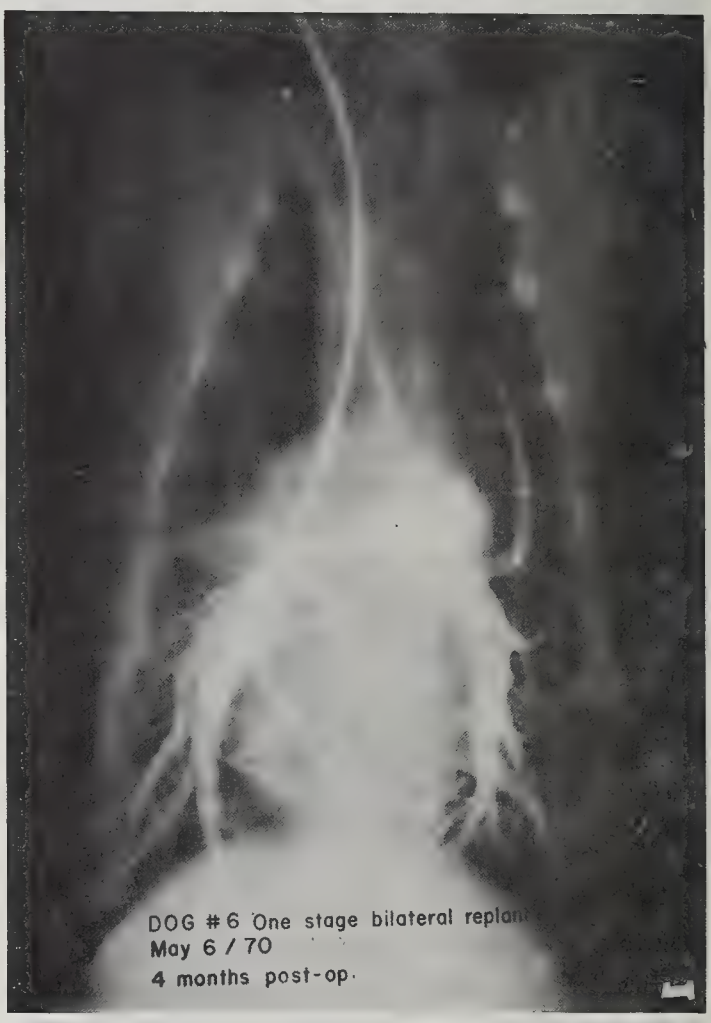


FIGURE 12d.

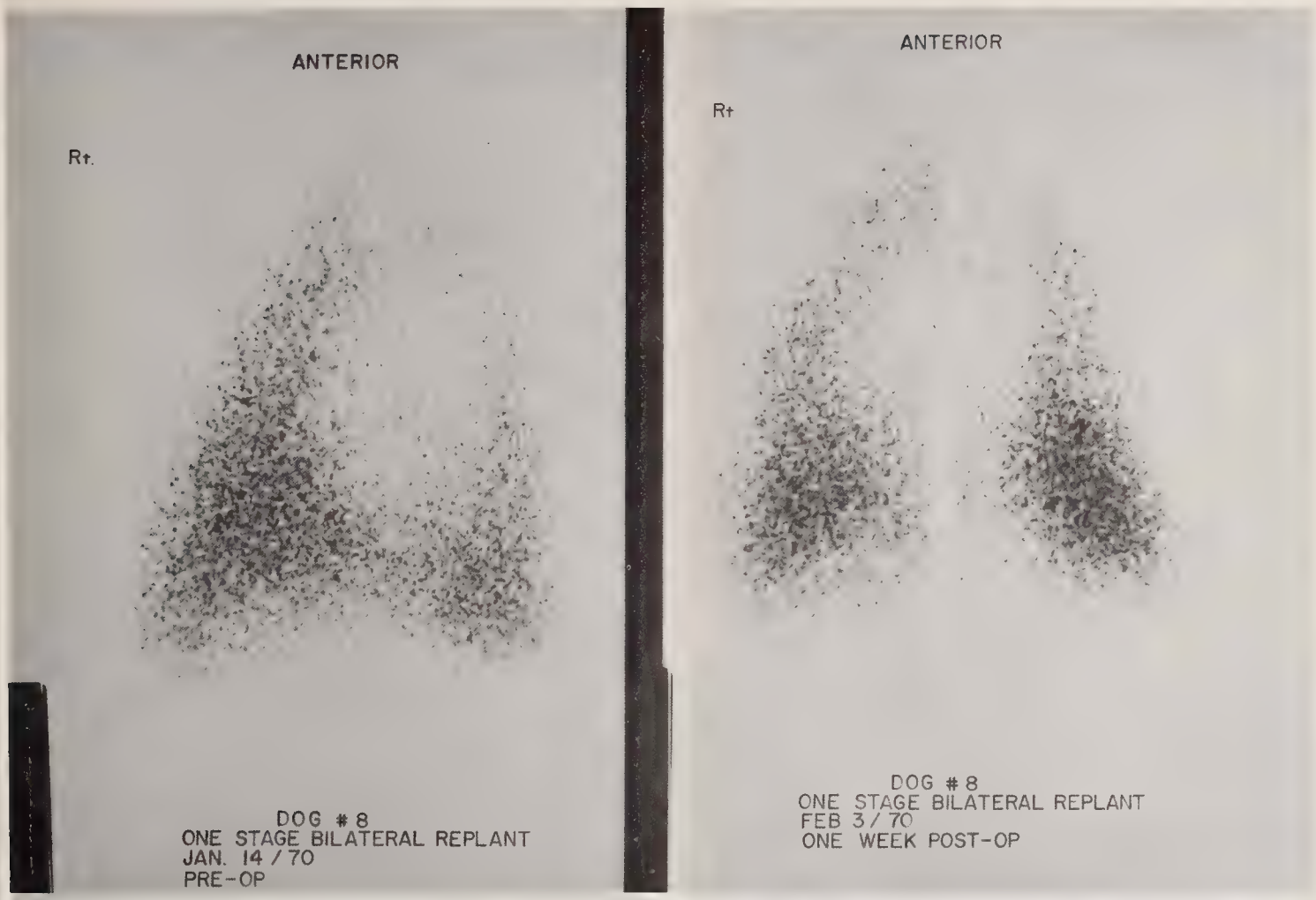


FIGURE 13.—Lung scans before and one week following bilateral lung replantation at one operation.

azathioprine, prednisone and mediastinal radiation. At the present time, we have one animal surviving 7 months after simultaneous bilateral lung allotransplantation, the immunosuppression therapy having consisted of prednisone and antilymphocyte serum (Figures 15 and 16).

Bilateral Lung Allotransplantation

The detection of lung rejection, when only one lung was allografted with one of the recipient's own lungs remaining intact, had always posed a problem. Since the animal could readily survive on his remaining lung, one had to resort to studies such as roentgenograms, scans, and differential bronchospirometry to estimate the function of the allografted lung.[68,69] Even with these measures, however, the precise functional contribution of the allograft lung was frequently in doubt. In marked contrast, the animal that has received two allotransplants is clearly surviving completely on allotransplanted tissue. Thus, we consider the bilateral simultaneous allotransplanted animal to represent the optimal model for future studies of immunosuppressive regimens to prevent lung allograft rejection. In such animals a routine monitoring of chest x-ray, lung scans and arterial blood gas values can give much valid information regarding the pulmonary function of the recipient. The risky techniques such as bronchospirometry which require anesthetization of the animal are thus avoided. As indicated above, there is at present no satisfactory method for identifying the degree or rapidity of rejection of a single lung allotransplant when the animal's own lung remains in place. The effectiveness of ventilation and perfusion can be measured by radioisotope scans, bronchospirometry and arteriograms, but it is difficult to differentiate atelectasis, pneumonitis, or ischemic

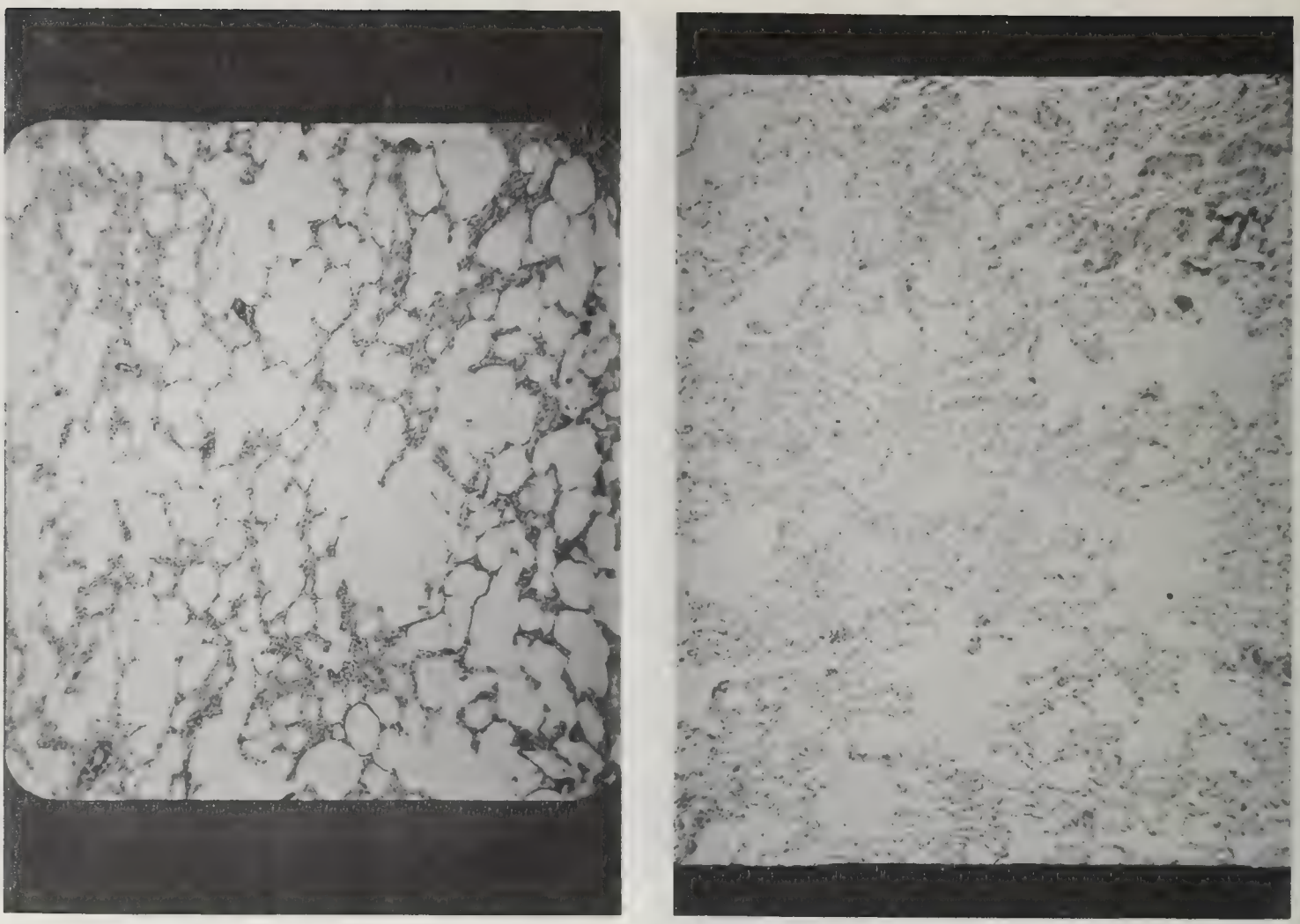


FIGURE 14.—*Left.* Normal dog lung. *Right.* Rejected dog lung allotransplant.

change from allograft rejection. Serial lung biopsies are impractical, and a precise method for assessing the degree of lung allograft rejection is badly needed.

It was demonstrated by Blumenstock and coworkers that well-matched animals rejected lung allografts less readily than did mismatched animals.[70]

Lung Allotransplantation for Treatment of Induced Pulmonary Hypertension

It was the large number of patients with respiratory insufficiency who first served to stimulate investigation of lung transplantation. Yet, an additional problem of less magnitude but of considerable clinical importance is that of pulmonary hypertension, often associated with cardiac lesions. Frequently, the patient has an intracardiac lesion which could be corrected satisfactorily with present operative techniques, were it not for the severe pulmonary hypertension which exists and which would result in an unacceptably high mortality rate following repair of the intracardiac lesion using cardiopulmonary bypass. Furthermore, some patients have severe primary pulmonary vascular hypertension, even in the absence of intracardiac lesions. This pulmonary hypertension can produce right heart failure and terminate in death. However, if lung allotransplantation were a safe and dependable procedure, many cases of pulmonary hypertension which would otherwise terminate fatally could be treated effectively.

The usefulness of lung allotransplantation for the reduction of induced pulmonary hypertension was studied in our laboratory.[71] First,



FIGURE 15.—Dog #BH-35 eight months after bilateral lung allografts performed at one operation.

pulmonary hypertension was produced by the repeated injection of a sterile suspension of barium into the pulmonary artery over a period of from 5 to 6 weeks. Three of the 22 dogs died during the period, but 19 dogs eventually developed pulmonary hypertension and underwent allotransplantation of the left lung. The 11 dogs that survived removal of the left lung and its replacement with an allograft were studied from 5 to 12 days postoperatively, but they showed no significant reduction in mean right ventricular pressure. It was concluded that allotransplantation of the lung did not afford relief from pulmonary hypertension during the first few days of the postoperative period. Such studies must be extended to identify the value of lung allotransplantation in long-term surviving animals. Weedn, Guenter and Greenfield [72] achieved somewhat longer survival of similar animals whose chronic pulmonary hypertension had been produced by the injection of glass beads.

LUNG TRANSPLANTATION IN MAN

It would be inappropriate to conclude this discussion without noting the accumulated experience with lung allotransplantation in man. The first human lung transplant was performed in 1963 following investigation of lung transplantation in several hundred dogs.[73] The problems met in the human experience were almost identical to those which had been encountered in the canine model, and transplantation of the lung in man was readily accomplished on the basis of the previous laboratory experience.

At the present time lung allotransplantation



FIGURE 16.—Chest x-ray in Dog #BH-35 (see Figure 15) 232 days after bilateral lung allografts. Arterial blood gas values are normal.

has been performed 28 times in human beings, with 23 of these cases having been reviewed by Wildevuur and Benfield.[74] In general, it has been established that the allotransplanted human lung functions well. Bilateral lung allotransplantation in man has shown beyond question that the respiratory requirements of the individual can be met by the allografts, provided that infection, atelectasis, and other complications do not impair the functional capacity of the transplanted lungs. The longest survivor of allotransplantation was Derom's case in Belgium.[75] This young man, whose opposite lung was essentially destroyed by silicosis, survived in fair condition for approximately 9 months. Our patient in 1963 lived 18 days and died from multiple problems which had been present and acknowledged preoperatively.[73] The lung functioned well until the terminal problems developed in the last 48 hours, and both gross and microscopic examination disclosed no evidence of rejection. Our second human lung transplant was performed in 1969, and this patient lived for approximately one month.[76] He died from invasive infection involving both the allotransplant and the opposite lung. Unfortunately, the commonest cause of death in lung transplantation has been overwhelming infection. The lungs are inevitably exposed to organisms from the air, and the essential immunosuppressive drugs also reduce the patient's defense against bacterial invasion. It has been clearly demonstrated that the human lung allograft can function effectively as an organ of respiration. What is needed now is better and more selective methods of immunosuppression, so that the ever-present bacterial organisms will not be given the capability of invasive infection which can kill the recipient. It is perhaps fair to say that lung transplantation and heart transplantation rest at approximately the level of progress which kidney transplantation enjoyed ten years ago. With increasing laboratory experience, it will be justifiable gradually to increase the clinical experience. Lung transplantation should eventually provide respiratory support comparable to renal support provided by the renal allografts at the present time.

SUMMARY

1. The dog model has proved highly valuable in the study of multiple facets and problems in lung transplantation.

2. Lung replantation in large numbers of dogs has identified the function pattern which can be expected of the replanted organ, in the absence of immunological changes which would be present with a lung allograft.

3. The function of a lung temporarily declines sharply following its replantation but function improves substantially over the next several weeks as pulmonary edema, pneumonitis and atelectasis subside.

4. Operative technique has now been refined to a point at which many dogs survive even immediate bilateral lung replantation.

5. Bronchial anastomotic complications of leakage or stricture are uncommon but do occur. Leakage has also occurred in several reported human cases.

8. Lymphatic communications are reconstituted across the bronchial anastomosis in from 7 to 12 days, and their substantial restitution parallels the subsidence of post-transplant pulmonary roentgen changes.

7. Intrinsic pulmonary nerves exhibit degenerative changes following lung replantation, but anatomical and functional recovery can be demonstrated months later in varying degrees in different animals.

8. Lungs replanted in puppies exhibit both anatomical and functional growth.

9. Lungs can be stored for hours, and provide substantial function on replantation. However, long-term storage success has not yet been achieved.

10. Bilateral lung replants, performed at the same operation, are now readily performed. This model compels the animal to survive completely on replanted tissue and, already, it has shown that pulmonary nerves are not essential for respiratory activity. However, the pulmonary nerves do contribute to the overall quality of respiration and lung blood flow.

11. Large numbers of lung allotransplantation experiments have been performed with the dog model. In general, the operation is readily accomplished and the lung functions. However, rejection eventually occurs in most animals,

regardless of the immunosuppressive agents used. The most commonly used moities are azathioprine (Imuran), prednisone, methotrexate, and antilymphocyte serum in various combinations.

12. The gross and microscopic changes of lung allograft rejection have been well described, but as yet no reliable objective and noninvasive method for detecting the stage of rejection is available.

13. The dog bilateral allotransplant operation now provides an excellent model for allograft study. The animal must survive solely on genetically dissimilar lung tissue, and all respiratory function is clearly derived from the transplants.

14. We have a colony of immediate bilateral lung allotransplant dogs surviving on prednisone and anti-lymphocyte serum suppression alone, without azathioprine which is relatively toxic to dogs. The oldest survivor has now gone eight months and is in excellent condition.

15. Based on experience previously gained with the dog model, approximately thirty human lung transplants have now been performed. Clinical lung transplantation should improve steadily as laboratory experience further extends knowledge in the field.

ACKNOWLEDGEMENTS

The work from our laboratories was performed in collaboration principally with Drs. Fikri Alican, Sadan Eraslan, Martin L. Dalton, Erol Isin, Yoshio Kondo, Kazuo Hino, and Mukadder Cayirli.

REFERENCES

1. Guthrie, C. C.: Bull. Hopkins Hospital 18:25, 1907. Cited in: *Blood Vessel Surgery and Its Applications*. Pittsburgh, U. Pittsburgh, 1959.
2. Demikhov, V. P.: *Experimental Transplantation of Vital Organs*. New York, Consultants Bureau Enterprises, 1962. pp. 130–135.
3. Staudacher, V. E., Bellinazzo, P., and Pulin, A.: Primary results in attempts at autoplastic reimplants and homoplastic transplants of pulmonary lobes. Chirurgia 5:223, 1970.
4. Metras, H.: Note preliminaire sur la greffe totale du poumon chez le chien. Compt. rend. Acad. Sci. 231: 1176, 1950.
5. Neptune, W. B., Weller, R., and Bailey, C. P. Experimental lung transplantation. Surg. Forum 3:379, 1952.
6. Juvenelle, A. A., Citret, C., Wiles, C. E., Jr., and Stewart, J. D.: Pneumonectomy with replantation of the lung in the dog for physiologic study. J. Thoracic Surg. 21:111, 1951.
7. Alican, F., and Hardy, J. D.: Lung reimplantation; effect on respiratory pattern and function. JAMA 183:849, 1963.
8. Allgood, R. J., Ebert, P. A., and Sabiston, D. C., Jr.: Immediate changes in pulmonary hemodynamics following lung autotransplantation. Ann. Surg. 167:352, 1968.
9. Blumenstock, D. A., and Kahn, D. R.: Replantation of the canine lung. J. Surg. Res. 1:40, 1961.
10. Duvoisin, G. E., Fowler, W. S., Payne, W. S., and Ellis, F. H., Jr.: Reimplantation of the dog lung with survival after contralateral pneumonectomy. Surg. Forum 15:173, 1964.
11. Sharma, A. N., Soroff, H. S., Bellas, A. E., Harrison, H. N., Sherman, J., and Deterling, R. A., Jr.: Experimental studies of the autotransplantation of pulmonary tissue. Surg. Gynec. Obst. 123: 295, 1966.
12. Bogardus, G. M.: An evaluation in dogs of the relationship of pulmonary, bronchial, and hilar adventitial circulation to the problem of lung transplantation. Surgery 43:849, 1958.
13. Brody, J. S., Fisher, A. B., Park, C. D., Hyde, R. W., and Waldhausen, J. A.: Factors influencing function of the autotransplanted lung. J. Appl. Physiol. 29:587, 1970.
14. Greenfield, L. J., Chernick, V., Hodson, W. A., and Brumley, G. W.: Alterations in pulmonary surfactant following compression atelectasis, pulmonary artery ligation, and reimplantation of the lung. Ann. Surg. 166:109, 1967.
15. Hardy, J. D., Eraslan, S., and Dalton, M. L., Jr.: Autotransplantation and homotransplantation of the lung: Further studies. J. Thorac. Cardiovasc. Surg. 46:606, 1963.
16. Trummer, M. J., and Christiansen, K. H.: Radiographic and functional changes following autotransplantation of the lung. J. Thorac. Cardiovasc. Surg. 49:1006, 1965.
17. Waldhausen, J. A., Daly, W. J., Baez, M., and Giammona, S. T.: Physiologic changes associated with autotransplantation of the lung. Ann. Surg. 165:580, 1967.
18. Waldhausen, J. A., Giammona, S. T., Kilman, J. W., and Daly, W. F.: Effect of transplantation of canine lung on pulmonary compliance and surfactant. JAMA 191:1002, 1965.
19. Yeh, T. J., Ellison, L. T., and Ellison, R. G.: Functional evaluation of the autotransplanted lung in the dog. Amer. Rev. Resp. Dis. 86:791, 1962.

20. Blades, B., Beattie, E. J., Jr., Hill, R. P., and Thistlethwaite, R.: Ischemia of the lung. Ann. Surg. 136:56, 1952.

21. Trimble, A. S., Kim, J. P., Bharadwaj, B., Bedard, P., and Wells, C.: Changes in alveolar surfactant after lung reimplantation. J. Thorac. Cardiovasc. Surg. 52:271, 1966.

22. Edmunds, H. L., Jr., Stallone, R. J., Graf, P. D., Sagel, S. S., and Greenspan, R. H.: Mucus transport in transplanted lungs of dogs. Surgery 66:15, 1969.

23. Marshall, R., and Gunning, A. J.: The long-term physiological effects of lung reimplantation in the dog. J. Surg. Res. 6:185, 1966.

24. Blank, N., Lower, R., and Adams, D. F.: Bronchial dynamics and the reconstruction of bronchial artery supply in the autotransplanted lung. Invest. Radiol. 1:363, 1966.

25. Neveux, J. Y., Bigon, J., Oancea, T., and Mathey, J.: Etude de la circulation arterielle bronchique. In: *Advances in Transplantation.* Baltimore, Williams and Wilkins, 1968.

26. Eraslan, S., Turner, M. D., and Hardy, J. D.: Lymphatic regeneration following lung reimplantation in dogs. Surgery 56:970, 1964.

27. Eraslan, S., Hardy, J. D., and Elliott, R. L.: Lung replantation; respiratory reflexes, vagal integrity, and lung function in chronic dogs. J. Surg. Res. 6:383, 1966.

28. Nakae, S., Webb, W. R., Theodorites, T., and Sugg, W. L.: Respiratory function following cardiopulmonary denervation with dog, cat and monkey. Surg. Gynec. Obst. 125:1285, 1967.

29. Nigro, S. L., Hirsch, E. F., Rams, J. J., Hamouda, E., and Adams, W. E.: Regression of the intrinsic nerves and other sequelae with reimplantation of the lung. J. Thorac. Cardiovasc. Surg., 54:815, 1967.

30. Howard, H. S., and Webb, W. R.: Respiratory paralysis following pulmonary denervation. Surg. Forum 8:466, 1958.

31. Eraslan, S., and Hardy, J. D.: Differential division of hilar tissue: Effects upon lung function in the dog. Dis. Chest 50:449, 1966.

32. Webb, W. R., Unal, M., Cook, W. A., Eraslan, S., and Hardy, J. D.: Growth and function of the transplanted lung in puppies. Clin. Res. 13:49, 1965.

33. Kottmeier, P. K., Cheng, C., Fitzgerald, J., Hochman, R., and Emmanuel, G. E.: Growth and function of the replanted lung in young dogs. J. Pediatric Surgery 4:66, 1969.

34. Hardy, J. D., Eraslan, S., and Dalton, M. L., Jr.: Autotransplantation and homotransplantation of the lung; Further studies. J. Thorac. Cardiovasc. Surg. 46:606, 1963.

35. Hino, K., Grogan, J. B., and Hardy, J. D.: Viability of stored lungs. Transplantation 6:25, 1968.

36. Blumenstock, D. A., Lempert, N., and Morgado, F.: Preservation of the canine lung *in vitro* for 24 hours with the use of hypothermia and hyperbaric oxygen. J. Thorac. Cardiovasc. Surg. 50:769, 1965.

37. Karlson, K. E., Garzon, A. A., Goldstein, S., Motlagh, F., and Torres, R.: Functional evaluation of immediately replanted and 24 hour preserved and replanted lungs after longer than 1 year. Bull. Soc. Internat. Chir. 29:175, 1970.

38. Largiader, F., Manax, W. G., Lyons, G. W., and Lillehei, R. C.: *In vitro* preservation of canine heart and lung. Arch. Surg. 91:801, 1965.

39. Largiader, F., Manax, W. G., Lyons, G. W., and Lillehei, R. C.: Technical aspects of transplantation of preserved lungs. Dis. Chest 49:1, 1966.

40. Gago, O., Benfield, J. R., Nigro, S. L., and Adams, W. E.: Left lower pulmonary lobe transplantation. JAMA 191:306, 1965.

41. Arnar, O., Anderson, R. C., Hitchcock, C. R., and Haglin, J. J.: Reimplantation of the baboon lung after extended ischemia. Transplantation 5: Suppl.: 929, 1967.

42. Haglin, J. J., and Arnar, O.: Physiologic studies of the baboon living on only the reimplanted lung. Surg. Forum 15:175, 1964.

43. Haglin, J. J., Telander, R. L., Muzzall, R. E., Kiser, J. C., and Strobel, C. J.: Comparison of lung autotransplantation in the primate and dog. Surg. Forum 14:196, 1963.

44. Alican, F., Cayirli, M., Isin, E., and Hardy, J. D.: Left lung replantation with immediate right pulmonary artery ligation. Ann. Surg. 174:34, 1971.

45. Hill, P. M., and Shaw, K. M.: Long-term survival of dogs after experimental pulmonary reimplantation and staged contralateral pneumonectomy. Thorax 23:408, 1968.

46. Lempert, N., and Blumenstock, D. A.: Survival of dogs after bilateral reimplantation of the lungs. Surg. Forum 15:179, 1964.

47. Slim, M. S., Yacoubian, H. D., Wilson, J. L., Rubeiz, G. A., and Ghandur-Manymneh, L.: Successful bilateral reimplantation of canine lungs. Surgery 55:676, 1964.

48. Faber, L. P., Pedreira, A. L. S., Pevsner, P. H., and Beattie, E. J., Jr.: The immediate and long-term physiologic function of bilateral reimplanted lungs. J. Thorac. Cardiovasc. Surg. 50: 761, 1965.

49. Trimble, A. S., Bharadwaj, B., and Bedard, P.: Successful bilateral lung reimplantation in the dog. Canad. J. Surg. 10:89,1967.

50. Alican, F., Cayirli, M., and Hardy, J. D.: Feasibility of simultaneous bilateral lung replantation Proc. III:524, 1971.

51. Alican, F., Cayirli, M., Isin, E., and Hardy, J. D.: One-stage replantation of both lungs in the dog. JAMA 215:1301, 1971.

52. ALICAN, F., CAYIRLI, M., ISIN, E., and HARDY, J. D.: Surgical technique of one-stage bilateral lung reimplantation in the dog. J. Thorac. Cardiovasc. Surg. 61:847, 1971.
53. ADAMS, W. E., GAGO, O., and ZAJTCHUK, R.: Use of azathioprine in homotransplantation of pulmonary tissue. Influence of size of graft in the maintenance of pulmonary function. Bull. Soc. Internat. Chir. 25:631, 1966.
54. BARNES, B. A., FLAX, M. H., BURKE, J. F., and BARR, G.: Experimental pulmonary homograft in the dog. I. Morphological studies. Transplantation 1:351, 1963.
55. CHRISTIANSEN, K. H., GROSS, R., FANFERA, F., STAINBACK, W. C., and TRUMMER, M. J.: Lung allografts with delayed opposite pulmonary artery ligation. Arch. Surg. 93:400, 1966.
56. FONKALSRUD, E. W., STEVENS, G. H., RANGEL, D. M., JOSEPH, W. L., YAKEISHI, Y., and TOCORNAL, J. A.: Physiologic evaluation of allogeneic canine lung transplants from living donors. J. Thorac. Cardiovasc. Surg. 57:607, 1969.
57. HARDY, J. D., ERASLAN, S., and WEBB, W. R.: Transplantation of the lung. Ann. Surg. 160:440, 1964.
58. HARDIN, C. A., and KITTLE, C. F.: Experience with transplantation of the lung. Science 119:3081, 1954.
59. HARDIN, C. A., KITTLE, C. F., and SCHAFER, P. W.: Preliminary observations on homologous lung transplants in dogs. Surg. Forum 3:374, 1952.
60. NEPTUNE, W. B., WELLER, R., and BAILEY, C. P.: Experimental lung transplantation. J. Thoracic Surg. 26:275, 1953.
61. BLUMENSTOCK, D. A.: Transplantation of the lung. Transplantation 5: Suppl.: 917, 1967.
62. BLUMENSTOCK, D. A., COLLINS, J. A., THOMAS, E. D., and FERREBEE, J. W.: Homotransplantation of the lung in dogs treated with methotrexate. Surg. Forum 12:121, 1961.
63. BLUMENSTOCK, D. A., GROSJEAN, O. V., OTTE, H. P., and MULDER, M. A.: Experimental allotransplantation of the lung. J. Thorac. Cardiovasc. Surg. 54: 807, 1967.
64. BLUMENSTOCK, D. A., OTTE, H. P., GROSJEAN, O. V., and Mulder, M. A.: Lung allografts in dogs treated methotrexate and antilymphocyte serum. Ann. Thorac. Surg. 6:33, 1968.
65. FLAX, M. H., and BARNES, B. A.: The role of vascular injury in pulmonary allograft rejection. Transplantation 4:66, 1966.
66. WARREN, B. A., and DE BONO, A. H. B.: The ultrastructure of early rejection phenomena in lung homografts in dogs. Brit. J. Exp. Path. 50:593, 1969.
67. HARDY, J. D., ERASLAN, S., DALTON, M. L., JR., ALICAN, F., and TURNER, M. D.: Re-implantation and homotransplantation of the lung: Laboratory studies and clinical potential. Ann. Surg. 157:707, 1963.
68. ALICAN, F., ISIN, E., COCKRELL, J. V., and HARDY, J.D.: One-stage allotransplantation of both lungs in the dog. (Submitted for publication).
69. VEITH, F. J., SINHA, S. B. P., TORRES, M., and RICHARDS, K.: Bilateral simultaneous canine lung allotransplantation. Ann. Surg. 174:48, 1971.
70. BLUMENSTOCK, D., WELLS, E., SANFORD, C., and DEGILLIO, M.: Allotransplantation of the lung in beagle and mongrel dogs prospectively typed for lymphocytic antigens. Transplantation 11:192, 1971.
71. SALEH, S. S., and HARDY, J. D.: Lung transplantation for treatment of pulmonary hypertension in dogs. Arch. Surg. 96:340, 1968.
72. WEEDN, R. J., GUENTER, C. A., and GREENFIELD, L. J.: Pulmonary allotransplantation in dogs with chronic pulmonary hypertension. Surg. Forum 21: 243, 1970.
73. HARDY, J. D., WEBB, W. R., DALTON, M. L., JR., and WALKER, G. R., JR.: Lung homotransplantation in man; report of the initial case. JAMA 186: 1065, 1963.
74. WILDEVUUR, C. R. H., and BENFIELD, J. R.: A review of 23 human lung transplantation by 20 surgeons. Ann. Thorac. Surg. 9:489, 1970.
75. DEROM, F., BARBIER, F., RINGOIR, S., GROLLY, G., VERSIECK, J., BERZSENYI, G., RAEMDONCK, R., and PIRET: A case of lung homotransplantation in man. (Preliminary report). J. Tijdschr. v. Geneeskunde 3:109, 1969.
76. HARDY, J. D., ALICAN, F., MOYNIHAN, P. C., TIMMIS, H. H., CHAVEZ, C. M., DAVIS, J. T., JR., and ANAS, P.: A case of clinical lung allotransplantation. J. Thorac. Cardiovasc. Surg. 60:411, 1970.

DISCUSSION

CHAIRMAN FRANK J. VEITH: I'd like to commend Dr. Hardy for a beautiful presentation. His laboratory has continued to make many outstanding contributions. Are there any questions or discussion?

PETER GEIS, Colorado General Hospital, Denver: Do you pretreat with antilymphocyte serum and prednisone before your homografts? And have you individualized the postoperative doses of prednisone?

DR. HARDY: We used to pretreat, but we found that it made little difference and we no longer do. At that time, we were also using Imuran. We now simply start prednisone and ALS treatment immediately after transplantation, and do not vary the dose.

DR. GEIS: What dose of prednisone do you use?

DR. HARDY: We've been using 5 milligrams per kilogram per day and decrease gradually over a two-month period.

CHAIRMAN: Do you think we will have to transplant one or two lungs in human patients?

DR. HARDY: I'll have to refer that question back to you. I recently read a manuscript of yours in which you suggest that experience with your patient showed we will not necessarily have to transplant both lungs. I myself don't believe anybody has proven that both lungs have to be transplanted, and I think we will continue to transplant one lung in human beings as long as we consider it reasonable to do so.

Let me point out that we can either transplant both lungs with the heart "en bloc" or transplant one lung and then the other lung. Dr. Haglin had a case in Minnesota in which he transplanted one lung into a patient and had the other one in the refrigerator; later in the day when the patient wasn't going to make it on one lung, Dr. Haglund transplanted the remaining lung on the other side, leaving the heart unmolested.

EXPERIMENTAL AORTO-CORONARY DIRECT REVASCULARIZATION IN THE DOG

Gerard S. Kakos* and H. Newland Oldham, Jr.**

The effects of aorto-coronary artery vein bypass graft on coronary hemodynamics in the presence and/or absence of coronary collateral flow were examined in an experimental canine model. In animals without coronary collaterals, construction of a bypass graft to the circumflex coronary artery produced no significant quantitative or qualitative changes in distal or adjacent coronary hemodynamics. Proximal coronary inflow was decreased, however, and functional occlusion occurred if the proximal segment had significant stenosis prior to bypass. In animals which underwent bypass six weeks after ameroid constriction of the circumflex coronary artery, significant collateral vessels were present. These were associated with an elevation of peripheral circumflex pressure and reduction of graft flow. Occlusion of the left anterior descending artery in these animals increased graft flow and reduced peripheral circumflex artery pressure. Conversely, after graft occlusion, left anterior descending artery flow increased and peripheral pressure decreased. The data document an initial decrease in aorto-coronary graft flow in the presence of extensive collateral vessels, but demonstrate a reduction in collateral flow following graft insertion.

INTRODUCTION***

Aorto-coronary artery direct revascularization has become a frequently employed method for treatment of occlusive coronary artery disease. Basic questions, however, regarding the effects of this procedure on proximal and distal coronary hemodynamics, ventricular functions, and the role of collateral vessels remain largely unanswered because of the difficulties encountered with producing a workable experimental model. This study was undertaken to produce canine preparations which would allow evaluation of these parameters and to evaluate both the acute effects of direct coronary artery bypass (vein graft) on proximal and distal coronary hemodynamics and the influence of collateral coronary vessels on the hemodynamic function of aorto-coronary bypass grafts.

*From the Departments of Surgery, The Ohio State University Hospitals, Columbus, Ohio and

**Duke University Medical Center, Durham, N.C.[2]

***Supported by N.I.H. Grants HE–90315, GMO–1709, and GM1539–06.

I. ACUTE CORONARY HEMODYNAMICS

Methods

Twenty-eight mongrel dogs, weighing between 20 and 26 kg were anesthetized with pentobarbital, 25 mg/kg, and ventilated through a mixing chamber with air and a 95% O_2–CO_2 mixture using a volume respirator. Through a left thoracotomy, a jugular or saphenous venous autograft was used to construct a bypass graft from the transverse aortic arch to the side of the mid circumflex coronary artery. The coronary anastomosis was performed during temporary circumflex occlusion without employing cardiopulmonary bypass or a local shunt. To prevent serious arrhythmias, lidocaine (40–50 mg) was given as a single, intravenous bolus prior to this manipulation. Ten of the 28 dogs survived with normal electrocardiograms and stable hemodynamic conditions and form the basis of this report.

Electromagnetic flow probes (Statham M 4001 flowmeter) were placed on the ascending aorta, proximal left anterior descending coronary artery (LAD), circumflex coronary artery distal to the vein graft anastomosis, and on the vein graft. Short, rigid catheters were inserted into the ascending aorta and distal circumflex coronary artery and attached to matched Statham P23 Db transducers for pressure monitoring. An adjustable occluder was positioned around the circumflex coronary artery 2.5–3 cm proximal to the vein graft insertion (Figure 1). Occluders were also placed about the vein graft and proximal LAD. Central aortic pressure, circumflex coronary pressure, ascending aorta flow, LAD flow, distal circumflex coronary artery, vein graft flow, and the electrocardiogram

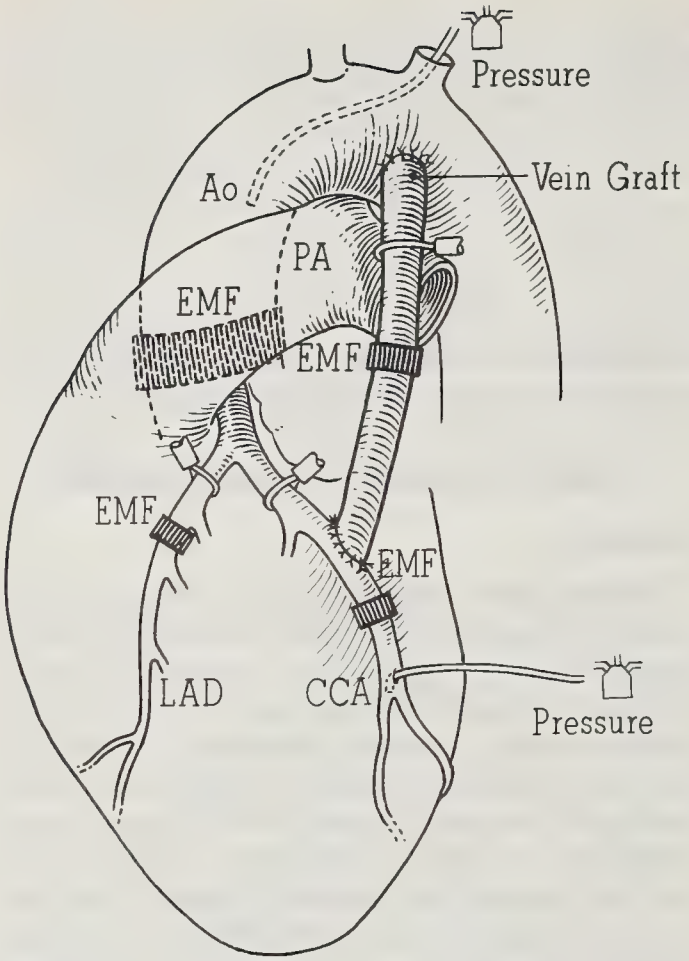


FIGURE 1.—Schematic representation of model
Ao—Aorta
LAD—Left Anterior Descending Coronary Artery
CCA—Circumflex Coronary Artery
EMF—Electromagnetic Flow Probe
Note: Pressure monitoring system and adjustable occluders.

were recorded simultaneously using a multichannel, direct writing oscillograph (Hewlett-Packard, Sanborn 7700 Series). Mean and pulsatile flow and pressure measurements were obtained after a 90 minute stabilization period.

Control values were gathered with the graft totally occluded allowing a normal source of coronary blood flow through the open circumflex coronary artery. Multiple measurements were then obtained under the following conditions: 1) graft open, proximal circumflex coronary artery open, 2) graft open, proximal coronary artery totally occluded, and 3) graft open, proximal circumflex artery subtotally occluded to varying degrees. Hyperemic responses, when induced, were allowed to regress prior to data collection.

The heart rate was not controlled, but data was measured only at rates of 140–160 beats per minute. Hematocrit and arterial blood gas analyses for pH, Po_2, and Pco_2 also were obtained.

Results

Hematocrit determinations varied less than 5% (±) in all animals while Po_2 was 140–240 mm Hg, Pco_2 20–34 mm Hg, and pH 7.32–7.39. Control mean flow through the unobstructed circumflex artery in all animals was 63 ± 15 cc/min (mean ± SD) and was 22 ± 2 cc/min in the LAD. During this period cardiac output was 2300 ± 300 cc/min and the mean aortic pressure 102 ± 10 mm Hg. Central circumflex coronary artery pressure (mean 100 ± 10 mm Hg) equaled aortic pressure. During temporary proximal circumflex artery obstruction, peripheral circumflex pressure was 10 ± 4 mm Hg, indicating the absence of significant collateral circulation.

Data was collected with the graft *open* and the circumflex coronary artery *unobstructed* to assess the source of flow to the distal circumflex artery. The graft supplied 57 ± 10% (mean ± SD) of the distal circumflex flow while 43 ± 10% entered through the unobstructed proximal circumflex coronary artery. Total distal circumflex flow was identical to control flow (graft occluded, circumflex open) and did not change when all flow was routed through the vein graft by totally obstructing the proximal circumflex vessel. Except for 4 to 8 msec acceleration delays noted in three animals in the latter group, the distal circumflex coronary artery phasic flow patterns were independent of the origin of flow and appeared identical to control data. During these interventions, LAD flow, cardiac output, and aortic, main circumflex and peripheral circumflex pressures remained constant.

An additional series of recordings was obtained while producing varying degrees of subtotal, proximal coronary artery stenosis by adjusting the proximal circumflex snare. With the graft occluded, proximal circumflex occlusion of less than 75% (cross-sectional area using

graded occluder) caused no coronary pressure gradient or appreciable change in distal circumflex coronary flow. Data obtained at these times mimicked results gathered with the previous manipulations and were the same as control values. Increasing the proximal circumflex stenosis, however, decreased distal circumflex flow by 35% to 83%, depending on the degree of stenosis, and produced pressure gradients across the obstruction of 10 mm Hg to 70 mm Hg. While maintaining the stenosis, the graft was opened and distal circumflex artery flow returned to the control value. Flow through the subtotally occluded proximal circumflex coronary artery was noted to decrease an additional 17% to 39%, and the bypass graft now supplied 74% to 100% of the total, distal circumflex coronary flow (Figure 2). Again, no quantitative or qualitative alterations in distal circumflex of LAD flow or in the cardiac output or aortic and coronary pressures were noted.

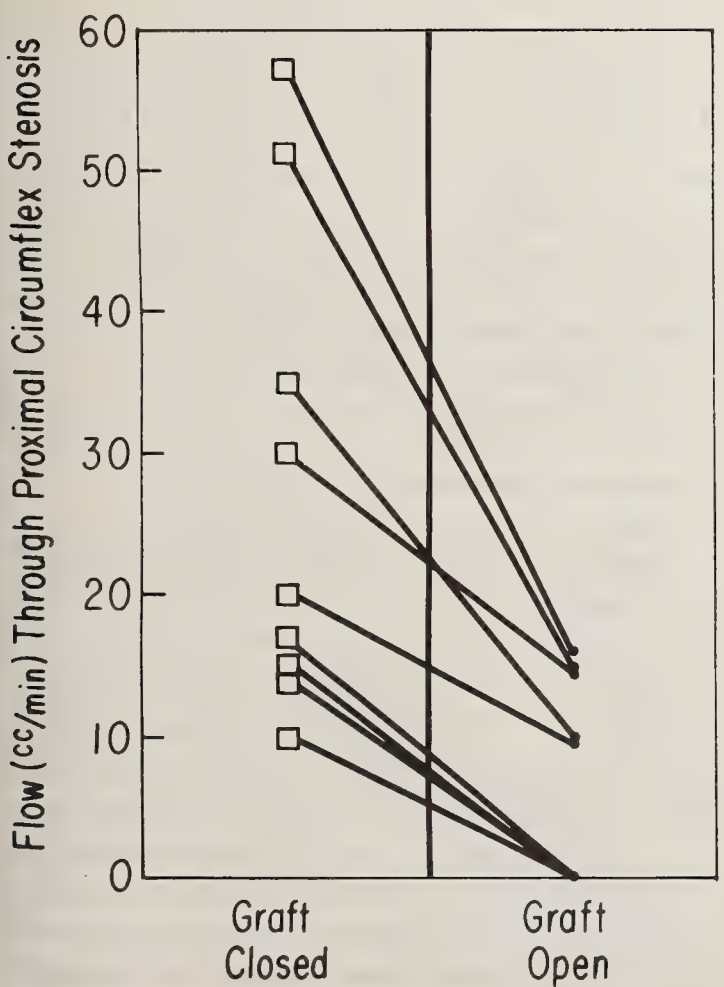


FIGURE 2.—Note marked decrease in proximal coronary artery flow through stenotic segment once the bypass graft is opened.

II. EFFECTS OF COLLATERAL CIRCULATION

Methods

Two additional groups, each containing 10 dogs weighing between 20 and 26 kilograms, were studied during identical pentobarital anesthesia and mechanical ventilation. Group 1 consisted of 10 animals with a jugular vein autograft acutely inserted between the aortic arch and the circumflex coronary artery (CCA), and ligation of the CCA proximal to the anastomosis. Again, the coronary artery anastomosis was performed during temporary circumflex occlusion without the use of cardiopulmonary bypass or a local shunt, using the identical surgical technique described in Part I. Group 2 was composed of an additional 10 dogs with an ameroid constrictor placed on the proximal CCA in standard fashion. Six weeks later a bypass graft was inserted between the aorta and the CCA distal to the site of the constrictor, and these animals were then studied. In both groups electromagnetic flow probes (identical to Part I) were placed on the ascending aorta, proximal left anterior descending coronary artery (LAD) and vein graft (Figure 3). Pressures were monitored in the ascending aorta, LAD and CCA using matched Statham P23Db transducers. Adjustable snares were placed around the graft and LAD for temporary occlusion. After 90 minutes of stabilization, simultaneous control pressures and flows were recorded on a multichannel direct writing oscillograph.

Mean peripheral LAD and CCA pressures were used to indicate collateral flow between these two vascular beds. Measurements were obtained after proximal occlusion of each vessel for one minute while recording the mean pressure in the respective peripheral arterial bed. Following release of this occlusion, measurements of reactive hyperemia were recorded. In both groups, graft flow and peripheral CCA pressure were recorded with the LAD first open and then totally occluded. Group 2 animals also had measurement of LAD flow and peripheral pressure with the graft both open and completely occluded. At completion of the experi-

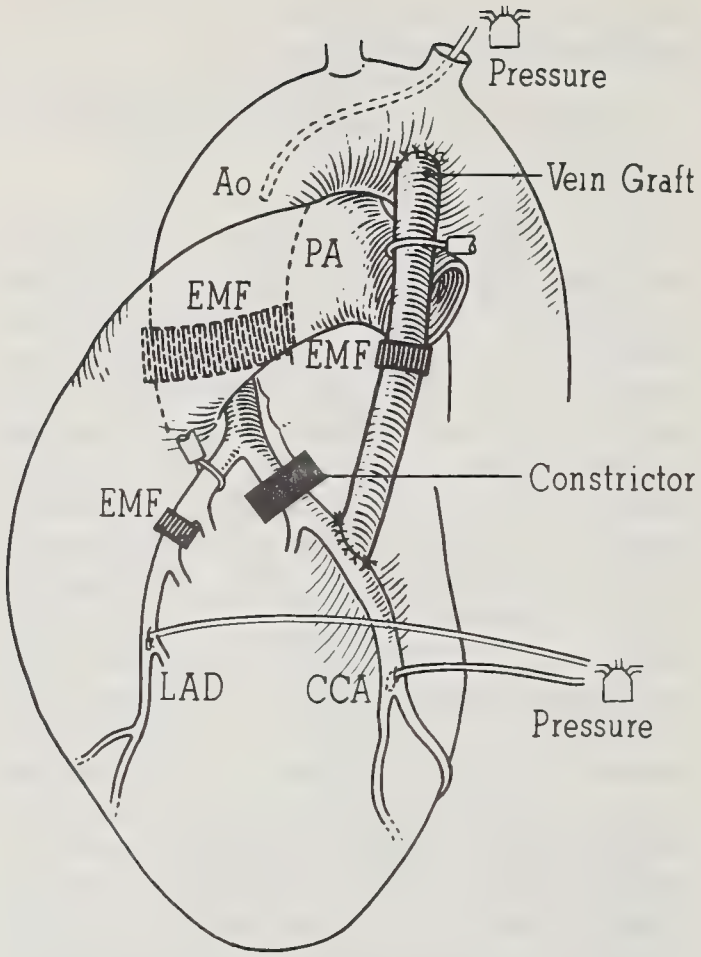


FIGURE 3.—Schematic representation of model
Ao—Aorta
LAD—Left Anterior Descending Coronary Artery
CCA—Circumflex Coronary Artery
EMF—Electromagnetic Flow Probe
Note: Pressure monitoring system and adjustable occluders.

ment, each heart was excised and examined grossly for visible collateral vessels. Postmortem arteriograms were performed on selected hearts from each group to demonstrate the presence or absence of collateral vessels.

Results

No visible evidence of collateral circulation was present in Group 1, and arteriograms with injection through the graft failed to demonstrate collateral channels between the CCA and LAD or right coronary artery. Average graft flow in these animals was 65 ± 17 cc/min (mean ± standard deviation), while LAD flow was 20 ± 3 cc/min. After temporary graft occlusion, mean peripheral CCA pressure measured 12 ± 4 mm Hg, and release was followed by the appearance of reactive hyperemia in each animal in the CCA bed. Occlusion of the LAD caused no demonstrable change in graft flow or in peripheral CCA pressure.

Collateral communications between the LAD and CCA were angiographically demonstrated in Group 2 by injection of the graft with opacification of both CCA and LAD. Varying degrees of right coronary artery filling were also seen in these animals. The proximal CCA was completely occluded by the ameroid constrictor in each animal. Control graft flow measured only 16 ± 8 cc/min, compared to 64 cc/min in Group 1. This value did not change during an average observation period of two and one-half hours. Occlusion of the LAD was accompanied by an increase in graft flow to 27 ± 13 cc/min ($p < 0.05$). Control peripheral CCA pressure was 62 ± 11 mm Hg contrasted to 12 mm Hg in Group 1. LAD occlusion was followed by a decrease of peripheral CCA pressure to 41 ± 17 mm Hg ($p < 0.05$). After release of LAD occlusion, flow in this vessel increased by more than two times in each animal indicating reactive hyperemia. These measurements demonstrated a marked decrease in graft flow with elevation of CCA peripheral pressure in the presence of collateral vessels. Occlusion of the LAD partially eliminated this collateral inflow to the CCA and was followed by a significant increase in graft flow and concomitant reduction in peripheral CCA pressure.

LAD flow in Group 2 during graft occlusion was 33 ± 7 cc/min compared to 20 cc in Group 1 indicating that flow from the central LAD was supplying both peripheral LAD and CCA vascular beds. Opening the graft reduced LAD flow to 27 ± 7 cc, but this decrease was not statistically significant ($p > 0.05$). Peripheral LAD pressure with the graft occluded measured 23 ± 14 mm Hg, or slightly greater than the value obtained for Group 1. Opening the graft elevated LAD peripheral pressure to 34 ± 17 mm Hg, an insignificant change ($p > 0.05$). After release of graft occlusion, nine of ten animals in Group 2 did not demonstrate CCA reactive hyperemia.

DISCUSSION

This study was designed to evaluate the effects that direct coronary artery revascularization might produce in the proximal, distal and adjacent coronary circulation in the presence or absence of collateral vessels. The canine model chosen proved both convenient and adaptable once mastered by persistent application of the outlined operative approach.

In Part 1, an experimental system without a large, functioning collateral circulation was chosen to obviate any mechanical alterations in the peripheral vascular bed[1] which might artificially influence the peripheral coronary collateral resistance and also to replicate the type of coronary collateral backflow currently reported in clinical investigations on this subject.[2] In addition, Khouri, et al.[3] have demonstrated experimentally that myocardial collaterals may become non-functional once forward flow is restored.

Coronary artery flow ultimately is controlled by the myocardial oxygen demand and by the availability of this oxygen to the myocardial tissue. After direct bypass improved ventricular function should be expected if myocardial depression existed and was due to ischemia.[4] In the presence of an adaptable peripheral coronary resistance, coronary artery bypass graft flow is dependent on the pressure gradient from the aortic graft to the recipient coronary artery. Assuming that both the anastomosis and the caliber of the graft are technically adequate, this pressure gradient is directly influenced by the timing of the pulse wave and arterial pressure contour transmitted through the graft, and by the flow and pressure pulse presented by the proximal portion of the bypassed coronary artery. Both pulse wave timing and pressure contour have been described as essentially normal when a short ascending aorta-to-coronary artery graft is utilized.[5] These findings were substantiated in this study with no evidence of delay or depulsation of the pressure or flow contours by the vein segment. The present investigation further demonstrated no alterations in flow, pressure or resistance in the coronary artery distal to the vein graft insertion as compared to the control (non-bypassed) state regardless of either the route of blood flow or the volume contributed by the vein graft or proximal coronary artery. Major changes, however, were produced in the proximal coronary artery flow after vein bypass. Bypass of a noncritically obstructed coronary reduced blood flow through this vessel by 50%. Concomitantly, the vein graft carried only one-half of its maximum load (excluding hyperemia). If the proximal coronary artery obstruction was critical, i.e. accompanied by a demonstrable decrease in distal flow and pressure, vein bypass functionally produced total or near total occlusion of this stenotic vessel. Under these circumstances, the paucity of collateral flow prevented any change in adjacent coronary artery flow as has been reported in selected clinical cases.[2,6] It would appear that proximal coronary artery flow and pressure are related directly to bypass graft flow. Because of this interdependence, the presence of a bypass graft will reduce flow in the coronary artery segment proximal to its insertion. In addition, if this segment contains a critically stenotic area, institution of bypass flow will produce either a functionally total occlusion or predispose this stenotic area to early thrombosis by severely limiting its volume flow. Distal coronary artery flow and pressure, nonetheless, remain totally independent of these relationships and are restored in a physiological manner by direct aorta to coronary artery revascularization.

In Part II, a system will extensive collateral circulation was chosen, again to redefine any effects they might have on the circulation within both the bypassed coronary artery and vein graft *per se*. Animals without demonstrable collateral vessels had functionally independent LAD and CCA vascular beds, and flow and pressure in a graft from the aorta to the acutely ligated CCA were not altered by temporary LAD occlusion. This was not true, however, in animals which had collateral communications with the CCA. After chronic CCA occlusion, the initial flow through a bypass graft inserted distal to the obstruction was only one-fourth that after acute obstruction. This was related to collateral flow into the CCA, as demonstrated by a high peripheral CCA pressure. Temporary occlusion of the LAD eliminated one source of collateral inflow and was accompanied by a

significant decrease in peripheral CCA pressure and a twofold increase in flow in the graft to the CCA. This residual CCA peripheral pressure of 41 mm Hg, as well as the failure of graft flow to elevate above 27 cc/min indicated an additional source of CCA collateral inflow, most likely from the right coronary artery. Khouri has observed a similar residual elevation of peripheral CCA pressure after temporary LAD occlusion, and also attributed this to communications with the right coronary artery.[3]

Occlusion of the LAD in the presence of collaterals was always followed by reactive hyperemia in the LAD peripheral bed. Nine of 10 animals, however, demonstrated no CCA reactive hyperemia after graft occlusion, possibly indicating lack of ischemia due to collateral inflow. Temporary occlusion of the graft was accompanied in each animal by an increase in LAD flow implying an immediate reduction in collateral flow after graft insertion. During the two and one-half hour period of observation, no further decrease in collateral flow could be demonstrated in this model. Khouri has recently demonstrated almost complete regression of flow through collaterals within three hours after release of CCA obstruction in the awake, intact dog.[3] Our preparation showed a decrease in collateral flow, but not a complete regression, and this difference may be due to the length of observation, the effects of the anesthetic agent, or again to a possible mechanical alteration within the collateral vessel wall.

Reversal of direction of flow through collateral channels has not been experimentally demonstrated. In the present study, peripheral CCA pressure was markedly decreased by clamping the LAD, but there was not a corresponding magnitude in reduction of LAD peripheral pressure after graft occlusion. This may indicate unidirectional transmission of pressure through collateral channels, but may be influenced by communications with the right coronary artery as indicated by the control value of LAD peripheral pressure of 23 mm Hg. Observations of graft flow and pressure made during insertion of human aorto-coronary saphenous vein bypass grafts have not clarified this situation.[2,6] Further human and animal observations are needed to determine the ability of flow in collateral vessels to reverse direction.

SUMMARY

The effects of aorto-coronary artery vein bypass graft on coronary hemodynamics in the presence and/or absence of coronary collateral flow were examined in an experimental canine model. In animals without coronary collaterals, construction of a bypass graft to the circumflex coronary artery produced no significant quantitative or qualitative changes in distal or adjacent coronary hemodynamics. Proximal coronary inflow was decreased, however, and functional occlusion occurred if the proximal segment had significant stenosis prior to bypass. In animals which underwent bypass six weeks after ameroid constriction of the circumflex coronary artery, significant collateral vessels were present. These were associated with an elevation of peripheral circumflex pressure and reduction of graft flow. Occlusion of the left anterior descending artery in these animals increased graft flow and reduced peripheral circumflex artery pressure. Conversely, after graft occlusion, left anterior descending artery flow increased and peripheral pressure decreased. The data document an initial decrease in aorto-coronary graft flow in the presence of extensive collateral vessels, but demonstrate a reduction in collateral flow following graft insertion.

REFERENCES

1. Schaper, W., Schaper, J., Xhonneux, R. and Vandesteene, R. The Morphology of Intercoronary Anastomoses in Chronic Coronary Artery Occlusion *Cardiovasc. Res.* 3:315, 1969.
2. Johnson, W. D., Flemma, R. J., Manley, J. C. and Lepley, D. The Physiologic Paramenters of Ventricular Function as Affected by Direct Coronary Surgery *J. Thoracic Cardiovasc. Surg.* 60:483, 1970.
3. Khouri, E. M., Gregg, D. M. and McGranahan, Jr., G. M. Regression and Reappearance of Coronary Collaterals *Am. J. Physiol.* 220:655, 1971.
4. Rees, G., Bristown, J. D., Dremkau, E. L., Green, G. S., Herr, R. H., Griswold, H. E. and Starr, A. Influence of Aortocoronary Bypass Surgery on Left Ventricular Function *N. Engl. J. Med.* 284:1116, 1971.
5. Wakabayashi, A., Beron, E., Lou, M. A., Mino, J. Y., da Costa, I. A., and Connolly, J. E. Physiological Basis for the Systemic-To-Coronary Artery Bypass Graft. *Arch. Surg.* 100:17, 1970.

6. MITCHELL, B., ADAM, M., LAMBERT, C., SUNGER, U. and SHIEKH, S. Ascending Aorta-To-Coronary Artery Saphenous V in Bypass Graft. *J. Thoracis Cardiovasc. Surg.* 60:457, 1970.

DISCUSSION

CHAIRMAN MICHAEL P. KAYE: Dr. Kakos' very interesting paper is open for discussion.

LANCE GOULD, Seattle: You mentioned that you increased the constriction to a critical point and then measured pressure fall and flow changes. How do you evaluate how much that constriction was? You indicated 75 per cent or so caused no coronary pressure gradient or appreciable change in distal circumflex coronary flow.

DR. KAKOS: Yes, 75 per cent is an approximation. I'm not sure it's within 10 per cent as a critical point. We used a graded constrictor, which was a double snare, and measured it. This figure refers to cross-sectional area and not diameter.

DR. GOULD: I see. Was the measurement done at the time you were doing the acute experiment?

DR. KAKOS: The final measurement was done afterwards. We marked the constrictor and subsequently measured it.

DR. GOULD: I see. It very nicely indicates what we found in humans with severe lesions who were studied post-operatively. If we do inject the bypass with dye, it very often appears to be physiologically constricted, although the openings may be there.

CHAIRMAN: Are there any further questions?

LLOYD PHILLIPS, University of Minnesota: Did you have an opportunity to evaluate the contribution of the right coronary artery to the collateralization of the left side? Also, did you have a chance to measure actual flows or volume distal to your occlusion on the left side?

DR. KAKOS: The first part of that I can answer by saying no. We did not produce another model with occlusion of the right coronary artery. That is being done now, and, in fact, some of these data are now becoming available. When you ask if we measured flow distal to the occlusion, do you mean during the period that the ameroid was occluding this vessel or afterwards by free flow into a container? No, we did not examine free flow into a container. I think that method may be fraught with some dangers because you remove all peripheral resistance and perhaps might not get an accurate measurement. We used peripheral coronary pressure as a time device for our collateral flow. And of course, we also monitored the left anterior descending coronary artery flow which was, I think, contributing the largest amount of blood to the system.

TOTAL CARDIOPULMONARY BYPASS WITH A MEMBRANE OXYGENATOR IN CALVES

M. P. Kaye and J. B. Pace*

Thirty-five Holstein calves weighing 65 to 110 kg were subjected to total cardiopulmonary bypass for periods up to 4 hours. Twenty-five underwent bypass with the Dow capillary membrane oxygenator. In ten of these animals, a filter was used on the oxygenator inlet. The remaining ten animals were subjected to bypass utilizing a bubble oxygenator. Anticoagulation was accomplished with an initial heparin dose of 4 mg/kg and an hourly dose of 1 mg/kg. Pressure drop across the oxygenators was measured for the Dow units. Blood samples were obtained at hourly intervals during bypass from the venous and arterial lines for pH, pO_2, pCO_2, hemoglobins and oxygen saturations. All animals had pre-operative biochemical and hematological surveys which were repeated on post-operative days 1, 3, 7 and 10. All were subjected to complete autopsies.

Our data indicate that two 3-liter Dow oxygenator units when used in parallel are capable of supporting a calf on total cardiopulmonary bypass when a filter is placed on the inlet end of the oxygenator. Use of the oxygenator without a filter resulted in formation of platelet aggregates on the oxygenator inlet tubesheet. Oxygen transfer rates at flows ranging from 2,500 cc to 5,000 cc per minute were comparable for Dow units with filters and bubble oxygenators. No significant alterations in hematology or biochemistry was noted consequent to perfusion with the membrane oxygenator. All animals perfused with the membrane oxygenator utilizing filters survived perfusion. Because of respiratory distress due to pneumonia, one animal was sacrificed on the first post-operative day. Nine animals had uneventful post-operative courses and were sacrificed on the tenth post-operative day. Four of ten animals subjected to perfusion with the bubble oxygenator expired during the course of bypass. An additional animal was sacrificed on the sixth post-operative day because of pulmonary complications. It is concluded that the hollow fiber oxygenator when used with a filter is capable of adequately supporting calves for four hours of total cardiopulmonary bypass.

INTRODUCTION

The work reported in this paper is based on the testing of a hollow fiber membrane oxygenator developed by Dow Chemical Corporation under direction of the Medical Devices Application Program, National Heart and Lung Institute.

In recent years numerous papers have described the advantages of membrane oxygenators over other oxygenators which function through a direct blood-oxygen interface.[1] The design and functional concept of the hollow fiber membrane oxygenator has been previously reported and has been shown to perform satisfactorily during partial bypass studies in dogs.[2] However, no information exists concerning the efficacy of this type of oxygenator in procedures requiring total cardiopulmonary bypass for periods approaching four hours. This manuscript describes the use of a disposable hollow fiber capillary membrane oxygenator for total cardiopulmonary bypass of four hours duration in calves and compares the long-term effectiveness of the hollow fiber device to a commonly used bubble oxygenator.

This study was designed to permit comparison of the gas transfer characteristics of the membrane oxygenator with data derived in an identical manner utilizing a standard bubble oxygenator in the perfusion circuit. In addition, the effects on blood constituents and animal survival of four hours of perfusion with both bubble and membrane oxygenators are compared.

METHODS

The hollow fiber membrane oxygenator used in these experiments (Figure 1) consisted of a bundle of 48,000 fibers spun from a siloxane-polycarbonate copolymer. At each end of the fiber bundle, the area between the fibers is filled with a potting material of silastic to form a tubesheet of approximately 2 centimeters in thickness. The sealed fiber bundle is surrounded by a lucite jacket which encloses the gas phase of the

* T & E Facility, MDAP, IIT Research Institute Chicago, Illinois.

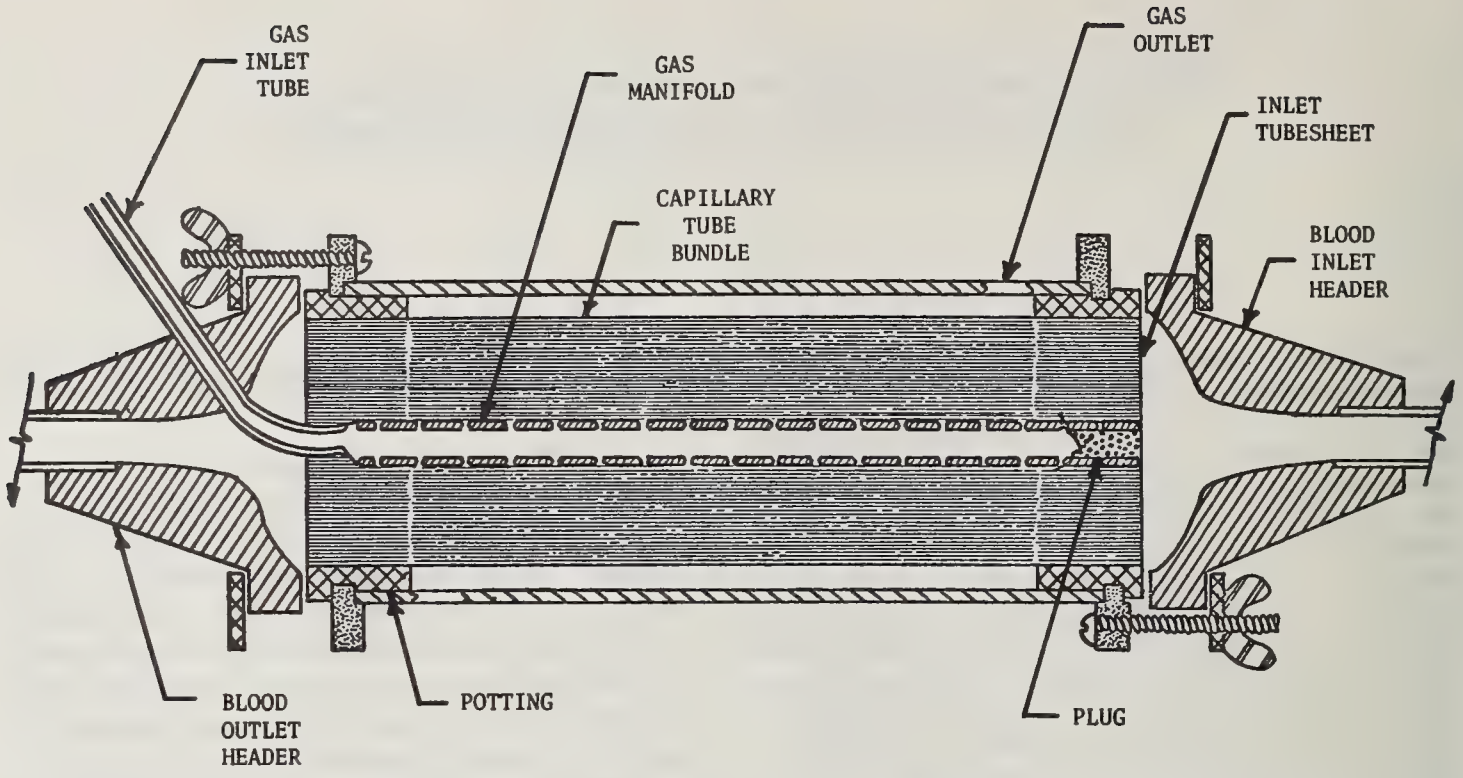


FIGURE 1.—Schematic representation of hollow fiber capillary membrane oxygenator. 48,000 fibers with a surface area of 4.2M² are used in the 3-liter units.

oxygenator. Oxygen enters a central manifold and is dispersed throughout the fiber bundles exiting through ports in the lucite jacket. At each end of the fiber bundle is a cone-shaped silicone header which directs the blood flow through the fibers. Sterilization of these units was accomplished by filling the fiber bundle and the headers with 0.5% formalin solution. In all experiments reported, two of the oxygenator units were connected in parallel and positioned in the vertical position.

Thirty-five calves ranging in weight from 65–110 kg were used in these studies. Three separate groups of perfusion experiments were carried out: (I) membrane oxygenator without filters (15 animals); (II) membrane oxygenator with filters (10 animals); and (III), bubble oxygenator (10 animals). A diagram of the circuitry used in the membrane oxygenator plus filter perfusions is illustrated in Figure 2. The circuitry consisted essentially of venous drainage into a 1-liter reservoir, a heat exchanger, and a roller pump which then pumps the blood through a filter and into the oxygenators. Blood from the oxygenators returns through the filter and into the femoral artery of the experimental animal. A similar circuit, but without filters, was used in the group I animals. Except for the reservoir and venous filters, a similar circuit was used in the perfusions with the bubble oxygenator.

Pressures across the filters were monitored continuously in all experiments using Statham P23dB transducers. In addition, pressures at both blood inlet and outlet of the membrane oxygenator were continuously monitored. Pressures were continuously recorded on a Beckman Dynograph.

In all animals, anesthesia was induced and maintained with fluothane, nitrous oxide and oxygen. Following intubation, the chest was opened through a right lateral thoracotomy in the fourth interspace. The azygos vein was ligated and the superior and inferior vena cava were isolated. The pericardium was opened and following intravenous administration of heparin (4 mg/kg), the inferior vena cava was cannulated through the right atrial appendage and the superior vena cava through a purse-string suture in the lateral wall of the right atrium. Arterial return to the animal was accomplished by cannulation of the right femoral

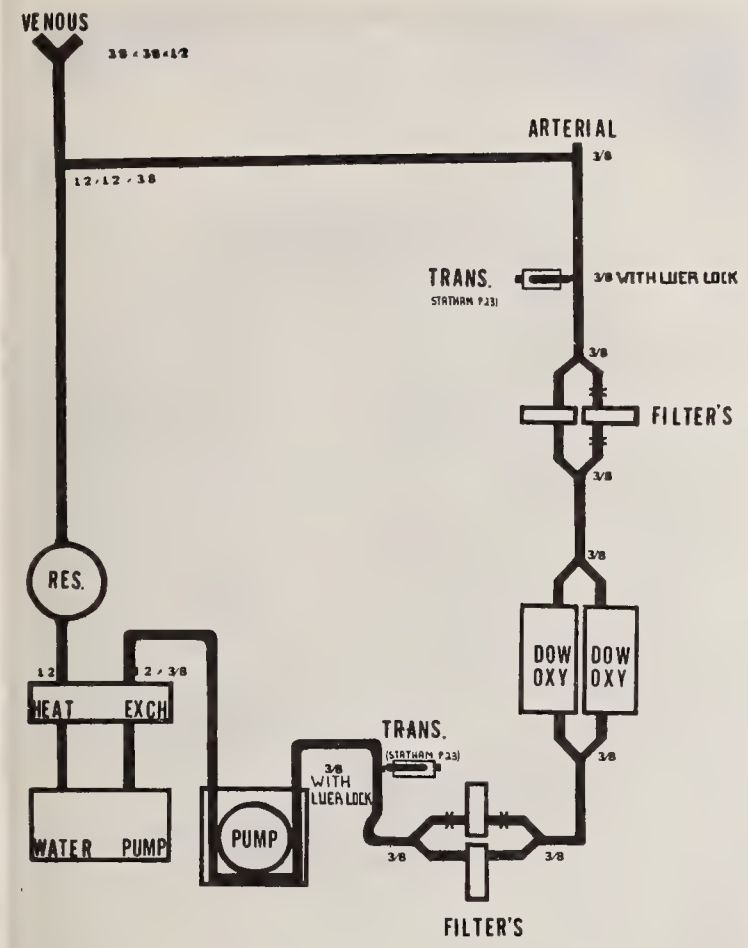


FIGURE 2.—Res. = Venous Reservoir; Heat Exch. = Disposable Venous Heat Exchanger; Pump = Roller Pump; Trans. = Pressure Transducer; Fractions refer to internal diameter of tubing.

artery. In all instances, the caval catheters ranged between sizes 36 to 40 and the arterial cannula was a size 18, 20 or 22. During the course of bypass, the lungs were inflated with helium and the endotracheal tube was clamped. Halothane was administered through the oxygenators with a 95% oxygen, a 5% carbon dioxide mixture and a gas flow rate of 30 l/minute. Heparin (2 mg/kg) was given hourly. Protamine sulfate in a dose approximately one half the total heparin dose was administered at the termination of the procedure.

Arterial blood samples for PO_2, PCO_2 and pH were drawn from the arterial pressure monitoring lines while the animal was under anesthesia and prior to bypass. After bypass was instituted, samples for pH, PO_2 and PCO_2 were obtained from inlet and outlet sides of the oxygenator immediately and then at hourly intervals for 4 hours. Additional samples were obtained for hematological and biochemical studies during the pre-operative period and on post-operative days 1, 3, 5 and 7. Table I lists the studies performed.

All animals in this study were subjected to complete autopsies at the conclusion of the experiment. The membrane oxygenators were flushed with a glutaraldehyde solution, then photographed and disassembled. After inspection, the headers and inlet and outlet tubesheets were also photographed.

TABLE I.—*Listing of Biochemical and Hematological Tests Performed on All Animals*

Hemoglobin
Hematocrit
Red Blood Cell Count
White Blood Cell Count & Differential
Platelets
Plasma Hemoglobin
Fibrinogen
Lactate
Bilirubin—Total and Direct
Creatinine
Potassium
SGOT
SGPT
LDH

RESULTS

In the initial group (I) of fifteen animals, it was noted that during the first 15–20 minutes of perfusion, the pressure gradient across the oxygenator increased rapidly. During the course of the four-hour perfusion, this gradient then continued to increase but at a very slow rate reaching an average maximum of 160 mm Hg at the end of 4 hours. Oxygenation during perfusion appeared marginally adequate and 10 of the 15 animals survived the experimental procedure. Examination of the oxygenators at the termination of the experiments revealed a significant deposition of platelet aggregates on the inlet tubesheets (Figure 3b) with occasional deposition of aggregates on the outlet tubesheets and both headers. Although it is almost impossible to quantitate the amount of platelet aggregate deposition, visual estimates of the amount of inlet tubesheet surface area covered provided adequate data to show a direct

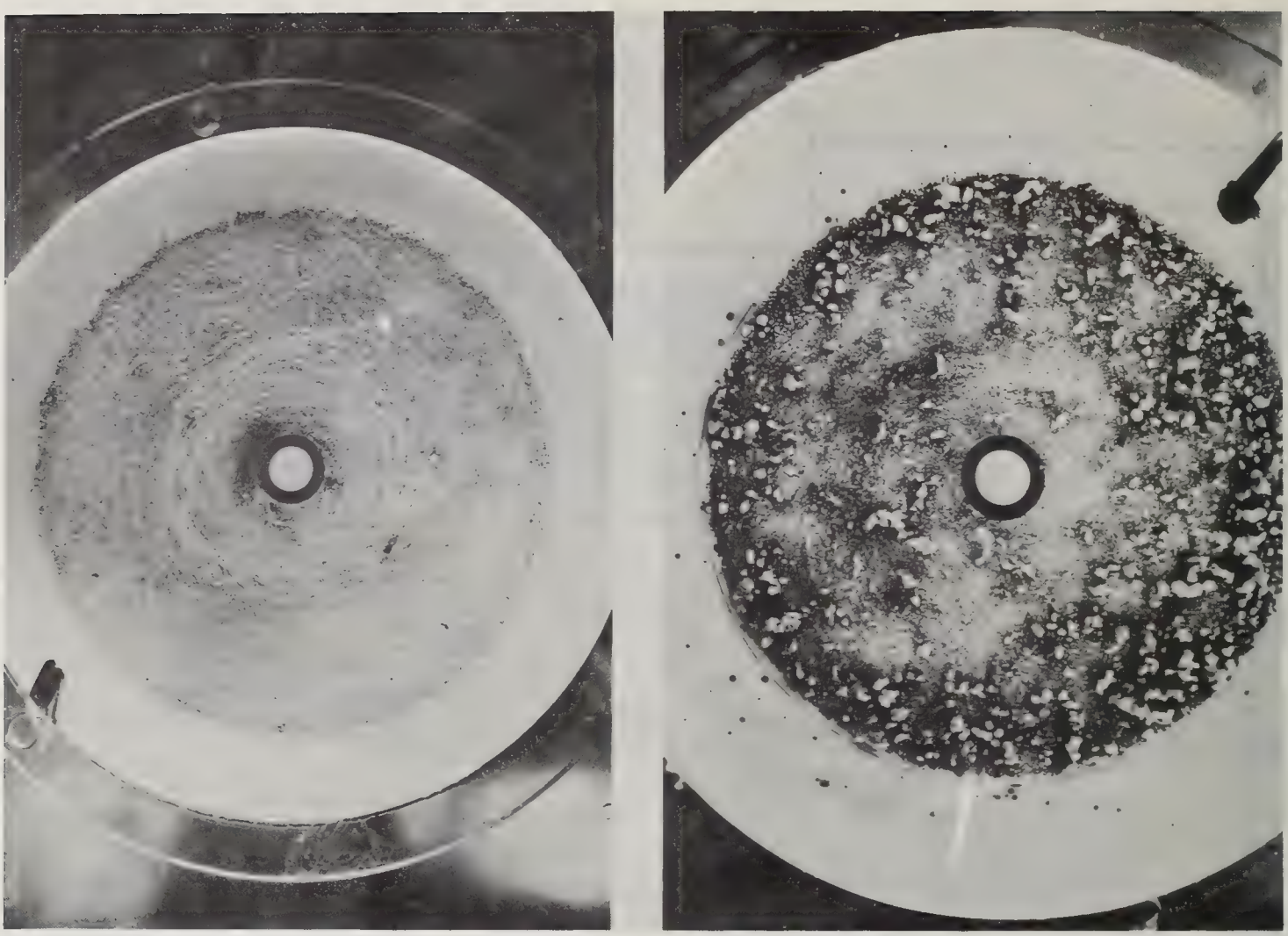


FIGURE 3A & 3B.—Photographs of inlet tubesheets taken following 4-hour perfusion. Debris on tubesheet is primarily platelet aggregates.

relationship between surface area occluded and increase in pressure gradient across the oxygenator.

These data indicated that the inlet tubesheet of the hollow fiber oxygenator acted as a filter with a pore size of approximately 200 microns. Since this filtering action resulted in the occlusion of capillaries and consequently a decrease in effective surface area of the oxygenator, all further experiments were conducted with a dacron mesh filter placed in the line on the inlet side of the oxygenator. Because of the occasional platelet aggregates noted on the outlet tubesheet, a filter was also placed in the outlet lines to insure patient safety.

In the second group of animals subjected to four hours of total cardiopulmonary bypass with the membrane oxygenator, filters were used in all ten experiments. There was no significant change in pressure gradient across the oxygenator from the beginning through the end of perfusion with the average final gradient being 105 mm Hg. Examination of the oxygenators after perfusion revealed them to be virtually free of thrombotic deposits as illustrated by the inlet tubesheet shown in Figure 3a. All animals survived the perfusion. Oxygen transfer and average results of blood gases and pH in the pre-operative period, at one hour of perfusion, and just prior to termination of perfusion are listed in Table II. Arterial pO_2 remained high throughout the procedure with complete saturation of the effluent blood. Oxygen transfer values ranged from 207 cc/min. to 223 cc/min. with inlet (venous) saturations

TABLE II.—*Summary of Blood Gases, Oxygen Transfer and Blood Flow of 10 Animals Subjected to Perfusion with Membrane Oxygenator*

		Average Values (10 Animals)		
		Pre-bypass	1 Hr.	4 Hr.
pO_2	Ven.	28	35	37
	Art.	354	286	304
Sat. %	Ven.	52	58	67
	Art.	99	99	99
pCO_2	Ven.	44	45	43
	Art.	30	38	36
pH	Ven.	7.44	7.29	7.36
	Art.	7.55	7.36	7.44
Flow (cc/min)			3907	4048
O_2 Trans. (cc/min)			223	207

ranging from 58 to 67% at flows of 3,900 to 4,200 cc/min.

These results substantiate the clinical impression that perfusion was adequate in all animals. When compared to the results obtained from perfusion of the group III animals (bubble oxygenator), it is apparent that oxygen transfer was comparable over a similar range of flow rates and inlet (venous) saturations, Table III.

Four of the animals in group III died during perfusion as a consequence of air embolism. Air embolism occurred in these animals because of inability of the bubble oxygenator to defoam adequately. In spite of the filters in the arterial line, considerable air was noted in the coronary arteries of these animals.

Hematological studies of the animals involved in this study revealed a decrease in hemoglobin consequent to perfusion (11.15 gm% pre-op.—9.98 gm% post-op.) as well as a decrease from the immediate post-operative value through day seven (9.51 gm% 1 day post-op.—7.0 gm% 7 days post-op). This decrease, however, was similar in both membrane and bubble oxygenator groups (Figure 4).

TABLE III.—*Summary of Blood Gases, Oxygen Transfer and Blood Flow of 6 Animals Surviving Perfusion with Bubble Oxygenator*

		Average Values (6 Animals)		
		Pre-bypass	1 Hr.	4 Hr.
pO_2	Ven.	21	37	36
	Art.	355	336	435
Sat. %	Ven.	43	60	62
	Art.	99	99	99
pCO_2	Ven.	35	47	46
	Art.	28	38	41
pH	Ven.	7.43	7.28	7.31
	Art.	7.45	7.34	7.35
Flow (cc/min)			3592	3453
O_2 Trans. (cc/min)			188	220

Platelet counts were decreased slightly in the immediate post-operative period but rapidly returned to normal for animals perfused with the membrane oxygenator. A similar directional change in platelet count was seen for animals perfused with the bubble oxygenator, but the decrease noted immediately following surgery was greater (Figure 5).

Changes in plasma hemoglobin levels were minimal following perfusion with the membrane oxygenator and compare favorably to levels noted following perfusion with the bubble oxygenator (Figure 6).

Studies of white blood cell counts, serum electrolytes, liver function, creatinine and serum enzymes revealed no changes not anticipated as a consequence of anesthesia, thoracotomy, cardiotomy and four hours of perfusion. Comparison of the results of these studies between group II and group III animals revealed no significant differences.

Post-mortem examination of animals surviving the ten-day experimental period revealed no significant findings other than those attributable to the surgical procedure.

CONCLUSIONS

The data presented indicate that two 3-liter Dow capillary membrane oxygenator units when used in parallel are capable of supporting a calf on total cardiopulmonary bypass for periods up to at least four hours. To permit a satisfactory perfusion, it is necessary that a filter be placed in the line on the inlet side of the oxygenator. Use of this oxygenator without a filter results in deposition of platelet aggregates on the oxygenator inlet tubesheet with consequent decrease in the number of functioning capillaries and hence effective surface area for gas transfer.

Oxygen transfer rates at flows ranging from 2,500 cc to 5,000 cc per minute were comparable for Dow units with filters and the bubble oxygenator. Because of low arterial pCO_2 noted in initial studies with one liter units, all experiments in this report were carried out using a mixture of 95% oxygen and 5% carbon dioxide

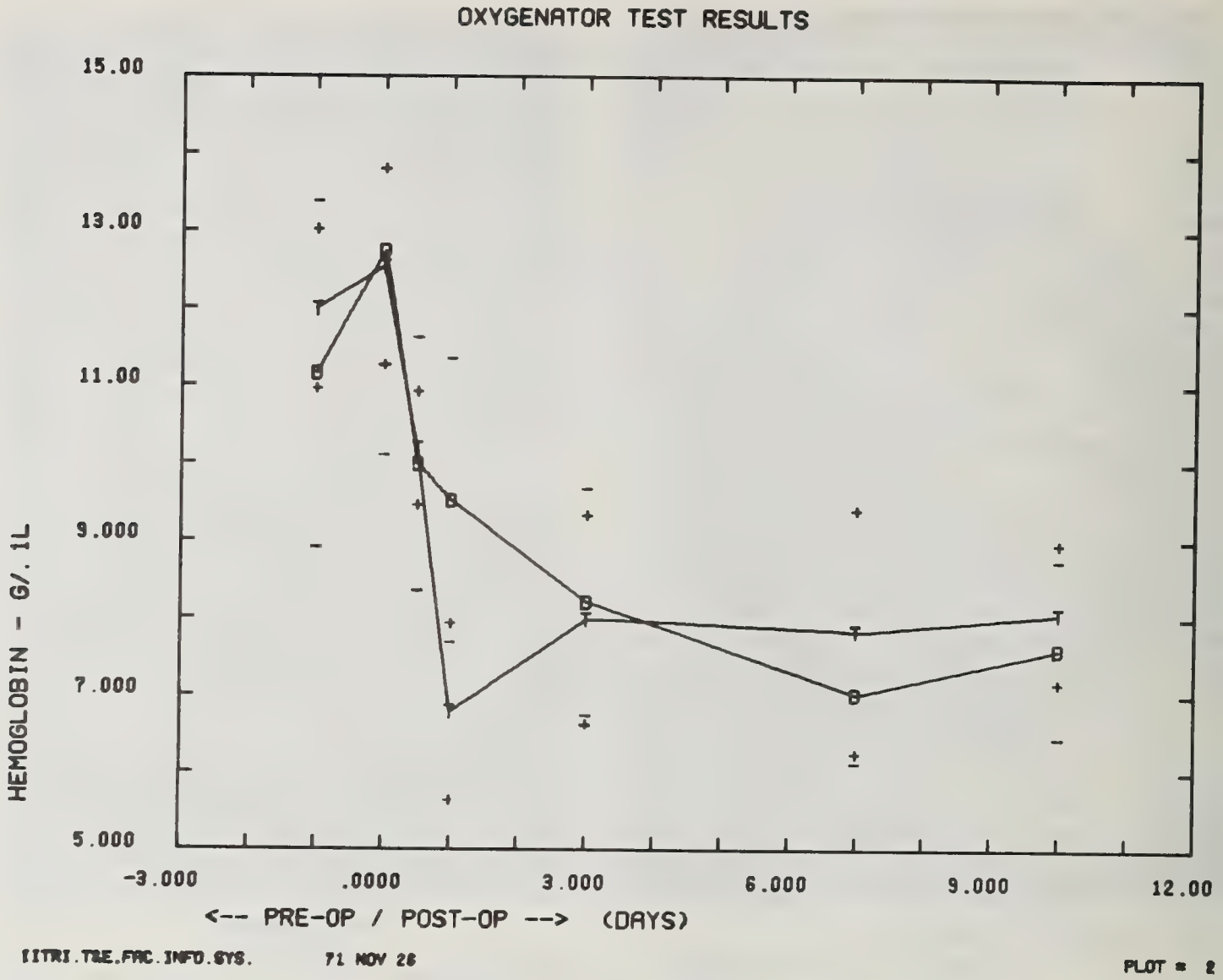


FIGURE 4.—Comparison of hemoglobin values from pre-operative period through post-operative day 10 for "D" (membrane) and "T" (bubble) oxygenators. + = standard deviation for bubble oxygenator; — = standard deviation for membrane oxygenator.

as the perfusion gas for the membrane oxygenator.

No significant alterations in hematology or biochemistry were noted consequent to perfusion with the membrane oxygenator. Decreases in platelet counts were of less degree than those noted with the bubble oxygenator. Hemolysis, as indicated by plasma hemoglobin levels, was minimal over the four-hour period during perfusion with the membrane oxygenator.

All animals perfused with the membrane oxygenator utilizing filters survived the perfusion. Four of ten animals subjected to perfusion utilizing the bubble oxygenator expired during the period of bypass.

Autopsy of animals surviving the ten-day experimental period indicated no significant pathology directly related to use of the membrane or bubble oxygenator.

From these studies, it is concluded that the Dow hollow fiber oxygenator when used with a filter is capable of adequately supporting calves for periods of at least four hours of total cardiopulmonary bypass. Because it is of low priming volume and eliminates a direct blood-oxygen interface, it offers certain advantages over the disc and bubble type of oxygenators.

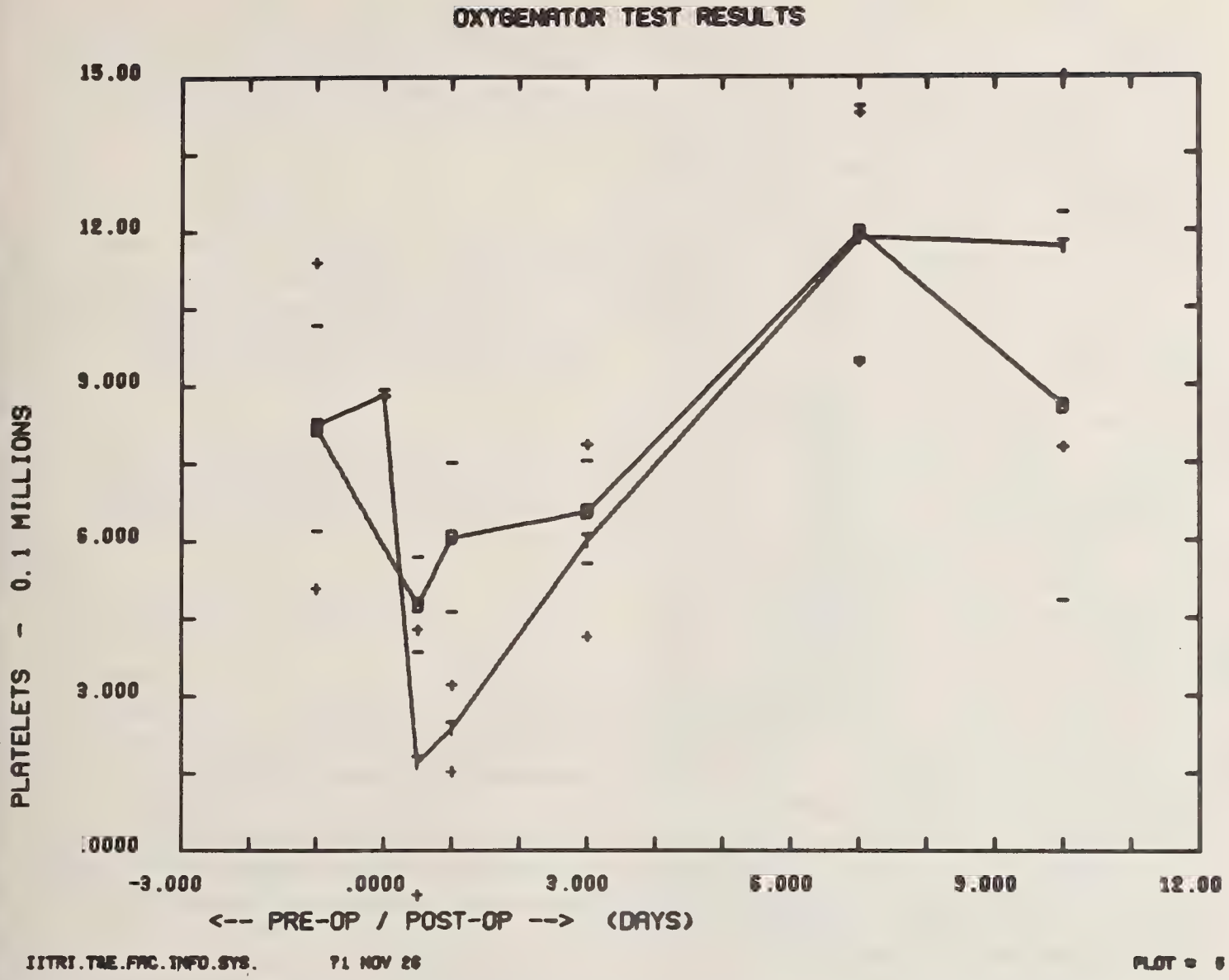


FIGURE 5.—Comparison of platelet counts of animals subjected to bypass with "T" (bubble) oxygenator and "D" membrane oxygenator.

SUMMARY

The use of the calf as an experimental animal has become commonplace in studies dealing with the artificial heart, its components and other heart and lung assist devices. Much pertinent information concerning surgical and anesthetic techniques in calves has been communicated to investigators as a result of this Conference. One of the most common problems dealt with was that of respiratory care of the calf subjected to anesthesia. Halothane is the apparent anesthetic of choice. The use of barbiturates was in most instances accompanied by a high mortality even when the very short-acting drugs were used. Large tidal volumes (1400–1600 ml) and high pressures (30–40 cm H_2O) were found to be necessary if a satisfactory post-operative course was desired.

During cardiac manipulation, it has been noted that the calf is prone to a high incidence of dysrhythmias. The use of lidocaine prior to manipulation has been successful in preventing these rhythm disturbances. In calves subjected to coronary occlusion for the production of cardiogenic shock, severe disturbances of rhythm (frequently ventricular fibrillation) are reported. The incidence of these arrhythmias has been decreased or abolished by infusion of insulin, dextrose and potassium.

The use of dogs as experimental animals in

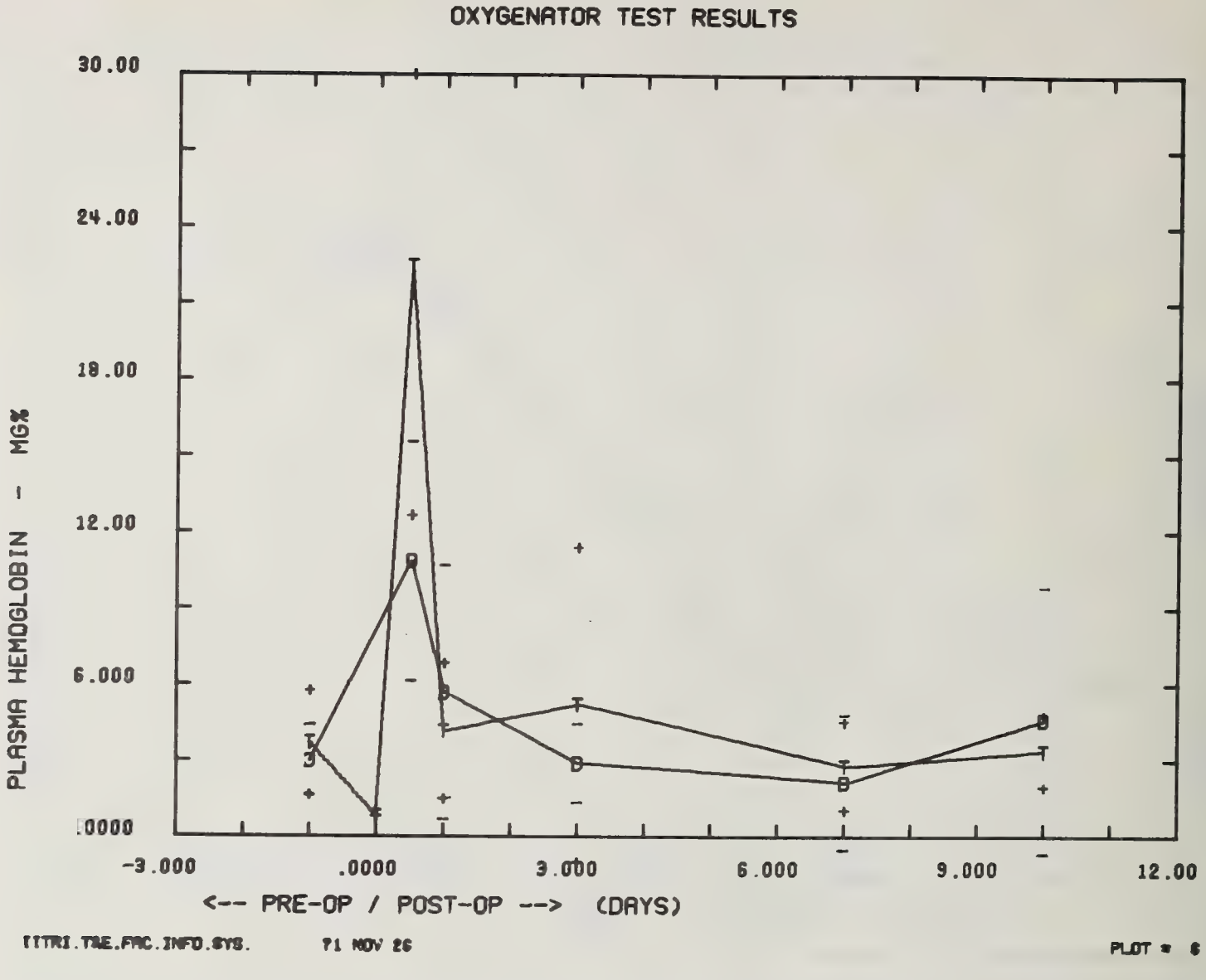


FIGURE 6.—Comparison of plasma hemoglobin levels of animals subjected to bypass with "T" (bubble) oxygenator and "D" (membrane) oxygenator.

studying cardiovascular phenomenon still predominants in most laboratories. Techniques for aorta-coronary artery bypass have been perfected and permit acute and chronic study of hemodynamic and functional effects of this procedure. It is emphasized that the most important factor in this procedure is practice until total coronary occlusion time can be reduced to less than 7 minutes.

In performing surgical manipulation on and about the heart, dissection frequently leads unknowingly to transection of the cardiac nerves and autonomic imbalance.

Finally, significant advances have been made in establishing colonies of non-human primates for experimental studies. Problems of husbandry, anesthesia, tranquilization, physical control while awake, prevention of disease transmission and post-operative care have been solved thus enabling the investigator to make a choice of the primate as an experimental animal based solely on the scientific merit of the species to be used.

REFERENCES

1. PIERCE, E. C., II, CORRIGAN, J. J., KENT, B. B., CATE, C. L., JOHNSON, R. L., and DREVES, A. E. Comparative Trauma to Blood in the Disc Oxygenator and Membrane Lung. Trans. Amer. Soc. Artif. Int. Organs 15:33, 1969.

2. DUTTON, R. C., MATHER, F. W., III, WALKER, S. N., LIPPS, B. J., JR., RUDY, L. W., SEVERINGHAUS, J. W., and EDMUNDS, L. H., JR. Development and Evaluation of a New Hollow Fiber Membrane Oxygenator. Trans. Amer. Soc. Artif. Int. Organs 27:331, 1971.

DISCUSSION

CHAIRMAN VEITH: Dr. Kaye's interesting paper is now open for discussion.

RICHARD E. CLARK, Washington University, St. Louis: What was the priming solution for both the circuits and were the volumes decreased?

DR. KAYE: The priming solutions were the same for both circuits. We used 2 units of freshly drawn calf blood and filled the remainder of the circuit with lactated ringers in all instances.

DR. CLARK: What was the volume?

DR. KAYE: The volume was approximately 2,500 cc. for both circuits.

JACK BLOCH, Cornell University Medical College, New York: I think everybody should know that Dr. Kaye has accomplished all this research after having this oxygenator at his disposal for less than 6 months. We took 2 years with our Landei-Edwards membrane oxygenator to get to the same point. I think a couple of things that we've learned might be of some help. The first point concerns the outflow filter in front of the membrane. We've had difficulty on long pump runs with the filter plugging up, and we now put two parallel filters in front of the unit (using only one at a time). If debris accumulates in one filter, we switch to the other without the hectic period of trying to change the single filter. Second, this unit was developed, given to the National Institutes of Health for testing, and then given to Dr. Kaye. There are groups of investigators around the country who have developed membrane oxygenators and similar devices who have been somewhat reluctant to follow this procedure, which I think is presently required of anybody with an NIH contract. I would ask that in the future, we set up some mechanism for the investigator who doesn't want to release his tool to the National Institutes of Health for independent testing. If the investigator has the facilities and the technical ability to do the testing himself, perhaps he should be allowed to do so.

DR. KAYE: Jack, thank you very much. I like to point out that we received these three liter units from the Dow Chemical Company after much of the preliminary work had been done at the CVRI. But the three liter units were not produced in this mold until June or July of this past year, and the 6 months from initiation of studies to our clinical trials is a very short period.

Although I did not mention it, our diagram shows two filters in the line, on the front and back end. Only one of these filters is used at any given time, and there is a clamp across the other. It's a safety device because filters do plug. During the course of these runs, we've had three of the filters plug up.

We currently have a series of investigations on the prolonged use of these oxygenators underway, and we have now pumped calves for periods of 4 and 5 days with one of these units doing partial bypass at rates up to 2,700 cc.

CHAIRMAN: You indicated that the reason you had the bad results with your bubble oxygenator bypasses was air embolism. But did you do a control where you used a filter and a bubble oxygenator?

DR. KAYE: Yes, we had filters in the arterial line with the bubble. But the foaming that we got from calf blood was so overwhelming that we couldn't defoam adequately through the oxygenator and it went right through the filters into the animal.

CHAIRMAN: Why did you use such high doses of heparin, 4 mg per kilogram?

DR. KAYE: We used this dose for a very poor but honest reason. Our clinical affiliate, the Rush Presbyterian St. Luke's Medical in Chicago, uses this dose routinely on its patients. Since we were going to do our clinical trials at their hospital, we thought we would use their protocol for bypass.

LOWELL HARMISON, National Institutes of Health: I'd just like to reflect on the comment about clinical testing. I think the philosophy of the Medical Devices Application Program and the National Heart and Lung Institute is given on page 97 of the official program and abstracts book for this conference. We are very much

aware of the time that's involved in clinical studies. And we do expect to be able to move directly to clinical studies because of the limitations of assessing effectiveness of equipment in animal models. I don't think that resources are at all affected in terms of projected clinical studies.

DR. CLARK: We have been studying two commercial membrane oxygenators for 2 years. But we have purposely done our studies with the dog because of its well known propensity to the problem of pump lung. Has the dog been used with the Dow lung?

DR. KAYE: Dutton and co-workers of CVRI published the results of their work with dogs in the ASAIO in 1971. The results were quite comparable to those of studies with the calf. We have not used the dog ourselves.

JERI DOBBS, University of Oregon Medical School: What type of bubble oxygenator did you use, and have you compared it with other membrane oxygenators?

DR. KAYE: We used the bag, the Travenol bubble oxygenator. We have not compared this membrane in our laboratories to other types of membrane oxygenators.

PRESERVATION OF ORGANS FOR TRANSPLANTATION

Theodore I. Malinin*

Simple hypothermia is the most commonly used method of preserving organs for clinical transplants. This method, despite its simplicity and low cost, has the disadvantage of not offering functional preservation for more than 12–24 hours. Preservation of organs for longer periods has many advantages. These include adequate immunological matching, selection and preparation of donors, and performance of the operation under other than "emergency conditions." The existing practical method of extracorporeal storage of whole organs for about 72 hours is hypothermic perfusion.

Perfusion is also necessary for the delivery of cryoprotective agents to the organs which are to be preserved by freezing, which appears to be the most feasible method for prolonged organ storage. Many attempts have been made to develop procedures for freeze-preservation of complex organs. None of these, so far, has produced organs which would sustain the life of experimental animals after transplantation. The difficulty with obtaining the desired results with freezing seems to lie not necessarily with the freezing of individual cells, but with the freezing of large masses of organized tissues. The delivery of cryoprotective agents in a sufficient concentration appears to be the main limiting factor in freezing attempts. This may be accounted for by the injury produced in the microvasculature by the introduction of cell-free solutions and high concentrations of cryoprotective substances which are potentially toxic. Organ perfusion with cell-free media requires the initial removal of the residual blood from the vascular tree of the perfused organ and its replacement with solutions which can deliver nutrients and oxygen to the tissue and remove metabolic waste products. The addition of red blood cells to the perfusion fluid has met with disappointing results, since the erythrocyte-containing solutions did not increase the extracorporeal life span of the perfused organ. Perfusion solutions which so far have provided organ survival in artificial environment include freeze-thawed and filtered blood plasma and hypertonic chemically-defined media. By use of these solutions the life span of perfused kidneys has been extended to five days and hearts to five-seven days. The damage sustained by organs preserved by these procedures has not been adequately studied and documented. Structural and functional alterations have been found in hearts, kidneys, and lungs subjected to artificial perfusion.

* Departments of Surgery and Pathology, University of Miami School of Medicine and Surgical Service, Veterans Administration Hospital, Miami, Florida.

INTRODUCTION**

Transplantation of organs in experimental animals has been performed routinely in many surgical laboratories since the demonstration by Carrel in 1910–11 of the practicality of canine renal autotransplantation.[1,2] Curiously, the problem of the extracorporeal preservation of organs intended for transplantation presented itself concomitantly with that of the surgical technique of vascular anastomosis. In the aforementioned experiments, reimplanted kidneys were perfused with and stored in Locke's solution for 50 minutes. The basic technique of organ preservation has not changed appreciably in the ensuing years. Short-term preservation still depends either on simple hypothermia or on a combination of hypothermia and perfusion. Long-term preservation of any complex organ in a viable state has not been accomplished thus far.

The dog remains the experimental animal used for the majority of organ preservation studies.[3] Some experiments have been performed on pigs.[4] The use of primates for the same purpose has gained prominence in the last decade.[5,6] Among small, inexpensive animals the rabbit has been employed for studies on renal preservation.[7]

Until relatively recently, kidneys used for transplantation experiments were preserved almost exclusively by hypothermia. In a dog, this method of preservation can provide a period of storage up to approximately 24-hours. The results with this method of storage, as applied to human kidneys, were likewise apparently satisfactory providing the total ischemic period

** Supported by the Office of Naval Research Contract N00014–67–A–0016.

of the organ was short and the proper storage temperature was used.[9] The advantage of simple hypothermia for the storage of organs is simplicity, low cost, and ease of transportation of the graft. However, the degree of injury sustained by the graft during simple hypothermic storage, although difficult to assay with precision, is not insignificant. With human cadaver transplants, approximately 50% of recipients required postoperative dialysis after receiving kidneys preserved by this method.

As contrasted to simple hypothermia, the other practical, widely used method which allows rapid recovery of function after short-term preservation of kidneys is hypothermic perfusion. In addition to permitting longer periods of preservation, perfusion helps to distinguish between functioning and non-functioning grafts prior to implantation.[8,10] However, it must be noted that the currently employed perfusion techniques do not allow for an indefinite maintenance of organs *in vitro*. The failure of organs to survive artificial perfusion beyond a certain point of endurance has been attributed to severe changes in the vasculature and the microvasculature of the perfused organ. Prolonged maintenance of perfused organs *in vitro* is also associated with a risk of bacterial contamination which increases greatly with each additional day of storage.[11]

It is clear that extracorporeal organ maintenance could be of increased value in clinical transplantation if the extracorporeal life span of the graft could be extended to weeks and months rather than hours. In the present state of technology this goal cannot be reached by perfusion. In fact, the only means by which indefinite organ storage can be anticipated is by freezing. Therefore, much experimental work on organ freezing has been performed. The retention of tissue and cell viability following freezing and thawing is not possible without the addition of cryoprotective agents. In the case of whole organs, these can be delivered only by perfusion.

Most tissues, when subjected to freezing in the presence of cryoprotective agents, survive on thawing, provided only small pieces are being frozen. The same holds true with small complex organs such as parathyroid glands, rat adrenals, chicken embryonic hearts, and intestinal segments.[12–14] Exactly opposite results are obtained with freezing of more complex, larger organs, even with the use of the same cryoprotective agents and freezing rates.[15,16]

TOLERANCE TO ISCHEMIA

The tolerance to ischemia varies greatly with individual organs, as it does with species of animals. Most parenchymatous organs, if transplanted after 1–2 hours of normothermic ischemia, will not function adequately. The adult human brain is uniquely susceptible to ischemic damage and is said to be irreversibly damaged by 10–15 minutes of ischemia. In the case of some organs, simple cooling will permit survival of isolated organs up to 8–24 hours.

The task of defining organ viability and various methods for assaying viability do not fall within the scope of this paper. For this purpose the reader may be referred to special publications devoted to the subject.[17–20] It may suffice to state that, for the purposes of the present discussion, the only meaningful assay of the viability of preserved organs is the resumption of function of such organs on implantation.

The function of organs rendered ischemic by the interruption of blood flow is obviously suspended until circulation is restored. The function of transplanted organs is usually impaired following completion of the vascular anastomoses and restoration of the blood flow. If the ischemic injury is not too severe this functional impairment is only transient. If, on the other hand, ischemic injury is severe enough to produce an irreversible change in an organ even before it is subjected to preservation procedures, the organ will not function on implantation.

The dog kidney seems to be uniquely resistant to ischemia, as compared to other organs and other animal species. Uncooled kidneys transplanted as long as one hour following ligation of the renal artery usually support the life of an experimental animal in the presence of the contralateral nephrectomy. The experience of the present writer indicates that rabbit kidney will not resume function after 20–30 minutes of ischemia. The kidneys of baboons and rhesus

monkeys apparently resume functioning after about one hour of normothermic ischemia, as do porcine kidneys.

In contrast to kidney, canine liver is extremely sensitive to ischemia, and usually becomes unsuitable for transplantation following 20–30 minutes of normothermic ischemia.[21,22] Primate liver is more resistant to ischemia, recovering after being subjected to ischemia for over an hour.[23] This suggests basic physiologic difference between canine and primate livers.

Morphological and ultrastructural changes are observed in the heart following even brief ischemia periods. After 8–12 minutes of total ischemia, De Gasperis et al. noted occasional mitrochondrial changes, while after 25–30 minutes they reported loss of cristae with clearing of the matrix of the mitrochondria, as well as loss of glycogen.[24] Forty-five to 50 minutes of ischemia led to mitrochondrial changes consisting of irregularity of shape, reduction in size with very close cristae, loss of matrix, transverse tubular dilation, indentation of the nuclear surface and loss of glycogen. The ischemic myocardium of dogs was studied by Denker et al.[25] who noted that critical membranous structures were altered as early as 20 minutes after the ligation of a coronary artery. However, these ultrastructural changes are apparently reversible, as their presence does not appear to prevent perfused hearts from contracting.[11] On the other hand, the lack of such changes did not assure the survival of transplants, as was demonstrated by Trunkey and co-workers.[26] This brings up the question of the full restoration of the organ function following transplantation. In transplanted kidneys, a decreased or even temporarily suspended function is still compatible with life. With hearts and lungs this is not the case, as even a brief significant impairment of function will lead to the death of the host. Any transplantation procedure with these organs must therefore provide for the immediate resumption of function from the implantation onwards.

Normothermic ischemic tolerances of lungs depend on whether the lungs are collapsed, inflated or ventilated. It was shown in experiments performed on dogs that the animals survived after one-half hour of ischemia with the lungs collapsed, after two hours of ischemia with the lungs ventilated, and after three hours when the lungs were inflated.[27] These data correlate well with previously-suggested safe upper limit of unmodified pulmonary ischemia of two hours.[28]

PRESERVATION OF ORGANS BY HYPOTHERMIA

The life span of an isolated organ can be increased several fold by simple cooling. Cooling is achieved either by exposing the organ to low ambient temperature or by flushing the organ with a solution of a desired temperature. For primate kidney preservation of several hours, the best results, as judged by their function following implantation, are obtained by simple cooling. Irrigation with small quantities of cold electrolyte solution produces some tubular damage, but has the added advantage of rapidly cooling the organ. Canine kidneys, when reimplanted into animals after six hours of storage at 4°C, will support the animal's life in virtually all cases. Kidneys stored 24 hours at the same temperature will support life in about 50% of animals. Only 25% of animals will survive after being transplanted with kidneys stored for 48 hours. Function does not return to kidneys transplanted after 72–96 hours of storage.[29]

Rhesus monkey liver has been successfully preserved by simple cooling for 3.5–5 hours.[23] Dog liver fails to function following two hours of simple hypothermia.

Hypothermia alone does not permit lung storage beyond a few hours. However, an increased storage time has been reported with hypothermia and hyperbaria. Garzon and associates[31] stored lung grafts at 4°C under oxygen at 2ATA for 24 hours. The pulmonary vasculature was flushed with lactated Ringer's solution. Seven animals were reimplanted with lungs preserved in this manner. These survived for a mean period of 21 months. There were no significant changes in the minute ventilation and oxygen uptake of these lungs, indicating that preserved reimplanted lungs maintained adequate functional capacity. However, the func-

tion of these procured lungs was not evaluated immediately after their implantation.

Hypothermia combined with hyperbaria, although apparently useful for short periods, has a time limitation providing a maximum effective storage period for kidneys of about 24 hours under ideal conditions.[31,32] Clear cut evidence of successful storage of hearts by this method is not recorded.[33] In order to differentiate between the physical effect of increased pressure and the increased oxygen saturation, kidneys were subjected to hyperbaria with either helium or oxygen by Samson and Murphy.[29] Kidneys exposed to helium demonstrated adequate creatinine and osmolar clearances. Deleterious effects of oxygen were manifested by increased renal vascular resistance to perfusion, and impairment of cortical perfusion as measured by the xenon clearance technique.

PERFUSION OF ORGANS

Various devices designed for perfusion of isolated organs have now been used for almost a century by physiologists and pharmacologists. Much of our present knowledge of physiology and the action of drugs on individual organs is derived from the use of these techniques. With the development of interest in organ transplantation, organ perfusion studies have gained a new impetus, stimulating the publication of new books and numerous papers devoted to the subject. It must be noted, however, that little has been accomplished in improving the actual technology of perfusion since the late thirties. All perfusion apparatuses are still utilizing the constant perfusion pressure or volume flow principle, which does not allow for the physiological regulation of blood flow. This inevitably causes damage to the perfused organs. The relatively short survival time of isolated perfused organs, the repeated observation of increased vascular resistance to perfusion as perfusion is continued and the demonstrable spasm of the vessels in isolated organs indicates that the microvascular system is the primary target which sustains damage during perfusion.

Several papers in which the results of various perfusion methods were compared, historical reviews given, and the various methods of perfusion described have been published in recent years.[34–37] Repetition of these efforts would serve little purpose.

Consideration of perfusion literature makes it quite clear that, despite all atempts to recreate the normal physiologic environment even under the best of circumstances, all existing perfusion systems are, in fact, unphysiologic. Most undesirable qualities of perfusion come both from the inflexibility of the delivery system and the deficiency of the perfusates. Blood cannot be used in most perfusion systems because of the inability of the erythrocytes and other cellular and non-cellular components to withstand mechanical injury and because of the clotting problems. This led to the utilization of blood components and the formulation of artificial chemically defined perfusion media. Among blood products, plasma was shown to be the most effective media by Humphries et al.[38] Cryoprecipitated and filtered plasma used by Belzer [36] allowed him to maintain functional, transplantable kidneys for several days. In the non-blood product perfusion media category, the best results were obtained with various tissue culture type media.[11]

When perfusion techniques are used for physiologic studies of isolated organs, the requirements are different from those of preservation. The aim in physiological studies is to reproduce the *in vivo* environment as closely as possible and to elicit maximal physiological function of the organ subjected to study. Therefore, physiologic studies are usually conducted at normothermic temperatures. In preservation attempts, temperatures which assure the longest survival of an organ are selected. Paradoxically, perfusion of organs at temperatures close to the freezing point does not extend the life span of an organ. The longest periods of "survival" of simian hearts perfused under identical conditions at different temperatures were obtained in the temperature range of 10–15°C. At these temperatures the hearts resumed contracting on rewarming after an average perfusion time of nine days. The hearts perfused at 4–6°C failed to contract on rewarming after 12 hours. Hearts perfused at room temperatures (22–25°C) pulsated, on an average, for 48 hours, while hearts perfused at normothermic

temperature (37°C) contracted for about 24 hours.[11]

The temperature dependence of the amplitude of contractions of isolated hearts was noted by Nawrocki in 1896.[39] The validity of his observations was easily confirmed by Gollan in a perfused dog.[40] He noted myocardial weakness in the region of 20°C. Above 20°C the heart contracted with sufficient frequency to eject the coronary thebesion flow from the left ventricle. At the time of highest contractility, the coronary flow and the myocardial oxygen consumption have undergone temperature-dependent decreases.

Some controversy still exists over the importance of pulsatile versus non-pulsatile flow. Little difference is observed in results between the two during the first eight-to-ten hours of perfusion of the kidney. However, kidneys which must be preserved for longer periods require pulsatile flow. Contractile ability of the heart is lost after non-pulsatile perfusion of over six to eight hours (personal observations). Thus, the necessity for pulsatile flow for long-term perfusion appears to be established.

To complete the discussion of asanguinous perfusion as a means of organ preservation, it is necessary to compare the results obtained by this method to those obtained by other methods. Preservation of functional dog kidneys for 72 hours by pulsatile perfusion at hypothermia has been achieved.[42] No other method of storage has offered equivalent-or-better results. In fact, when the functions of autografted canine kidneys preserved for 24 hours by perfusion or by simple hypothermia alone were compared under identical experimental conditions, perfused kidneys functioned satisfactorily while kidneys preserved by hypothermia alone did not.[37] The experience with simian hearts is similar.[11]

With animal kidneys, perfusion flow characteristics indicate the patency of vascular bed and have been relied upon by many investigators. More precise tests of physiologic function can also be performed.[7,8]

FREEZING AND CRYOPROTECTIVE AGENTS

Ideally, long-term preservation methods should be simple and should provide for an indefinite storage of tissues. Freeze-preservation, when possible, meets these criteria. It has made a marked impact on tissue culture, on experimental hematology and on the animal breeding industry. It has also provided for indefinite storage of non-viable tissues.

Since the advent of refrigeration, biologic materials have been preserved at temperatures below 0°C.

The use of refrigeration for preservation of biochemical entities has created a general feeling that the lower the temperature, the longer a specific biological activity will be maintained. This is not the case when physiologic integrity of organs must be preserved. In practice, all too often specimens are frozen at random with temperature selection being based not on the knowledge of phenomena associated with freezing, but simply on the availability of a given type of refrigeration. Changes which are produced in tissues, cells and biologic fluids as a consequence of crude freezing methods are significant.

Lowered temperatures will decrease metabolic rate, but cold, by itself, usually does not kill cells. However, irreversible injury is produced by freezing and is caused by a multiplicity of factors which may include chemical injury, mechanical injury, dehydration, and metabolic aberrations resulting from storage at temperatures where some enzymes continue to function.

To achieve the near cessation of biochemical activity, it is necessary to store the tissues well below 0°. Many investigators have for years stored their material at —79°C, the melting point of easily-obtainable solid carbon dioxide. However, for long-term storage of tissues, it is necessary to maintain temperature below —120°C. Only at such temperatures can the damage caused by the gradual growth of ice crystals be avoided. These temperatures are easily obtained by the use of liquidified gases, such as nitrogen and helium.

A review of events associated with cooling, freezing, storage at sub-zero temperatures, and thawing of tissues places emphasis on the significance not only of heat transfer, but also of various associated phenomena. The change from a colloidal to a solid state does not occur instantaneously but takes place within a few

minutes or hours, depending on the size of the specimen and the temperature differential between the specimen and the cooling medium.

In a majority of instances the cell death in mammalian tissues may be simply the result of failure to survive crystallization of freezing. Ice crystals may be formed both extracellularly and intracellularly. Whether ice crystal formation is extracellular or intracellular is, at least in part, the function of rate of freezing. Ice formation is essentially extracellular when the drop in temperature is relatively slow. During ultrarapid freezing (many degrees per second), microscopic ice crystals form within cells and throughout the suspending fluids. Crystal size is also a function of the rate of cooling. However, after the solid state has been reached, recrystallization or growth of crystals may occur, depending on the temperature of storage.[43]

To avoid lethal damage by freezing, tissues are exposed to cryoprotective compounds prior to freezing. Although the exact mechanism of action of agents in the prevention of freezing injury is not well understood, these agents have been successfully used in freezing of certain mammalian tissues. After thawing, the viability of such tissues has been adequately demonstrated both *in vivo* and *in vitro*.

The first chemical agents which prevented freezing damage when added to tissues were ethylene glycol and glycerin glycerol. These were later supplemented by dimethyl sulfoxide.

Since the introduction of ethylene glycol, glycerol and dimethyl sulfoxide (DMSO) as cryoprotective agents, polyvinyl pyrrolidone (PVP), dextran, hydroxyethyl starch and magnesium have been added to the list. However, no systematic screening, pharmacological testing and evaluation of cryoprotective properties of new potential cryoprotective compounds on different biologic systems has been undertaken. Thus, old, familiar agents—glycerol and dimethyl sulfoxide—remain the most widely used and studied agents.[43]

Toxicity of cryoprotective compounds has been customarily measured by alteration of cell structure and cell growth. Toxicity in whole organs can be reliably estimated only by measuring function on transplantation. The growth of HeLa cells in cultures was severely inhibited by exposure to DMSO in "cryoprotective" concentrations, suggesting that a large proportion of cells did not survive the treatment. Concomitantly, with the reduction of the growth potential cytopathologic changes were noted in treated cells.[44]

In addition to direct toxic injury cryoprotective agents produce osmotic changes when introduced in the vascular tree. Karow[45] states that 2.IM glycerol or DMSO, when introduced in the vasculature, exert approximately 2,100 mOsm of osmotic force attracting water to the vascular compartment. Such osmotic imbalance exists as long as it takes the cryoprotective agent to equilibrate between the vascular and the interstitial fluid.

In whole hearts perfused with 2.IM DMSO in Ringer's solution, initial "phasic contracture" which relaxed gradually, presumably due to osmotic equilibration, was observed. No marked changes in the contractility of hearts perfused with media NCTC 135 prepared with hypertonic balanced salt solution, and up to 15% DMSO were noted by the author. However, when the concentration of DMSO exceeded 15%, the heart stopped contracting abruptly (personal observations).

Compared to isolated cell suspensions, whole organs are indeed very complex structures. When fluids of various tonicity are introduced in the vasculature of organs, equilibration takes place primarily between the interstitial fluid and the intravascular fluid, as is evident from perfused organs in which edema can be produced by perfusion with hypotonic and "isotonic" solutions. In such organs the interstitial spaces are widely dilated and, if edema is severe, the delicate supportive connective tissue is disrupted. In the heart, the myocardial fibers themselves remain relatively unaffected. Edema forms quickly, as judged by an extremely rapid gain in weight of about 20–30% of either hearts or kidneys perfused with the so-called isotonic solutions.

Edema in perfused organs is reduced rapidly when 2 to 3x "isotonic" concentration of inorganic salt is used in the perfusate. Autotransplanted kidneys perfused with these solutions sustain life of rabbits and dogs in the presence of an immediate contralateral nephrectomy, and

the perfused hearts continue to pulsate until inorganic salt concentration is increased to 3½–4X "isotonic" levels. Gain and reduction of weight can be likewise produced by varying concentration of cryoprotective agents in the perfusate.

Consideration of the response of whole organs to cryoprotective agents will not be complete without the mention of the physiologic peculiarities of some organs, as exemplified by the heart. The effect of cryoprotective agents on cardiac rat muscle has been studied by Karow.[45] He found dextran, DMSO and magnesium to be effective cryoprotective agents, and glycerol an ineffective one. He attributed the ineffectiveness of glycerol to its high toxicity to rat heart.

To be effective a cryoprotective substance must be in contact with the cells to be preserved, and must be present in sufficiently high concentrations. It appears, in view of vascular-interstitial space equilibration, that the latter condition is not met in the case of cells in organs perfused with the usual concentrations (7–15%) of commonly used cryoprotective agents. If this is, in fact, the case, the failure to protect whole organs from freezing damage can be easily explained. If, on the other hand, commonly used cryoprotective agents are introduced into the vascular tree in higher concentrations, irreversible injury due to direct toxic effects takes place. Thus, the major problem of freeze-preservation of organs remains unsolved. It is still impossible to prolong extracorporeal life span of an extirpated organ by freezing. Hearts will not survive cooling below −20°C despite the use of cryoprotective agents. Furthermore, even at this temperature resuscitation cannot be achieved after storage of 20 minutes. Kidneys will not survive freezing. On the other hand, the last two decades witnessed an impressive progress in cryobiology. Whole blood, bone marrow, lymphocytes, protozoa, dispersed cells and micro-organisms are frozen and thawed routinely with remarkable success. Even though results of studies with freezing whole adult heart are far from encouraging, results obtained by Conway et al.[46] and Rey[47] with embryonic hearts indicate that there is probably no inherent peculiarity of cardiac tissue which would prevent it from being frozen and resuscitated on thawing.

At this point, I would like to suggest a different approach to studies dealing with organ preservation. The detailed study of the vascular response to perfusion, introduction of cryoprotective agents, and to cooling and freezing itself must form the basis of this approach. The response of microvasculature to freezing and thawing must be studied directly in the organs being frozen, and not anatomically dissimilar structures.

SUMMARY

The research in organ preservation has been stimulated by a sheer pragmatic need for organ transplants used in an attempt to extend life in critically ill patients. It is hoped that this will continue to simulate advances in the field of organ preservation.

REFERENCES

1. Carrel, A. Doppelte nephrektomie und reimplantation einer niere. Arch. f. Klin. Chir. 28:379, 1909.
2. Carrel, A. The ultimate result of a double nephrectomy and the reimplantation of one kidney. J. Exp. Med. 14:124–125, 1911.
3. *Organ Preservation and Perfusion.* (Ed: J. C. Norman), Appleton-Century-Crofts, New York, 1968.
4. Lokkegaard, H., Gyrd-Hansen, N., Iverson Hansen, R., Hasselager, E., Rasmussen, N., and Rasmussen, F. Kidney preservation with pulsatile and non-pulsatile hypothermic serum perfusion. Acta. Med. Scan. 188:245–255, 1970.
5. *Experimental Medicine and Surgery in Primates.* (Eds: E. I. Goldsmith and J. Moor-Jankowski), Ann. N.Y. Acad. Sci. 162, Art. 1, 1969.
6. *Medical Primatology.* (Eds: E. I. Goldsmith and J. Moor-Jankowski), S. Karger, Basel, 1971.
7. Hollerman, C. E., and Malinin, T. I. Functional evaluation of isolated kidneys perfused with bloodless fluids. In: *Microcirculation, Perfusion and Transplantation of Organs.* (Eds: T. I. Malinin, B. S. Linn, A. B. Callahan and W. D. Warren), Academic Press, New York, pp. 313–326, 1970.
8. Malinin, T. I., and Hollerman, C. E. Evaluation of renal function in perfused rabbit kidneys. J. Surg. Res. (In Press) 1972.
9. Starzl, T. E. What's new in surgery transplantation. Surg. Gynec. Obstet. 130:316, 1970.
10. Belzer, F. O., Reed, T. W., Pryor, J. P., Countz, S. L., and Dunphy, J. E. Cause of renal injury in kidneys obtained from cadaver donors. Surg. Gynec. Obstet. 130:467–477, 1970.

11. Malinin, T. I. Pulsatile perfusion of hearts with bloodless fluids. In: *Microcirculation, Perfusion and Transplantation of Organs.* (Eds: T. I. Malinin, B. S. Linn, A. B. Callahan, and W. D. Warren), Academic Press, New York, pp. 349–367, 1970.
12. Holst, H. I., Hamilton, R., and Lehr, H. Fresh and frozen canine parathyroid autografts: Functional evaluation by mortality rates. Cryobiology 4:258, 1968.
13. Gonzales, F., and Luyet, B. Resumption of heart beat in chick embryo frozen in liquid nitrogen. Biodynamica 7:1–5, 1950.
14. Hamilton, R., and Lehr, H. B. Survival of small intestine after storage for seven days at —196°C. Cryobiology 3:375, 1967.
15. Manax, W. G., Bloch, J. H., Longerbeam, J. K., Eyal, Z., and Lillehei, R. C. *In vitro* freezing of whole organs. Cryobiology 1:157, 1964.
16. Wheeler, T. E., Thomas, E. D., and Malinin, T. I. A method for freezing rat kidney in situ. Transplantation 2:534–540, 1964.
17. Malinin, T. I., and Perry, V. P. A review of tissue and organ viability assay. Cryobiology 4:104–115, 1967.
18. Abbott, W. M. Viability assay as applied to the cryopreservation of hearts and kidneys. Cryobiology 5:454–462, 1969.
19. Sell, K. W. Viability assay of organized tissues and organs. Cryobiology 4:133–135, 1967.
20. Dolan, M. F. Viability assays—A critique. Fed. Proc. 24:S275–279, 1965.
21. Goodrich, E. D., Welch, H. F., Nelson, J. A., Beecher, T. S., and Welsh, C. S. A report of homotransplantation of canine liver. Surgery 39:244, 1966.
22. Buckberg, G. D., Tocornal, J. A., Byfield, J., et al. Functional and structural alterations during hepatic ischemia. In: *Organ Perfusion and Preservation.* (Ed. J. C. Norman), Appleton-Century-Crofts, New York, pp. 285–303, 1968.
23. Slapak, M. M., Chir, M., Braddeley, M., Wexler, M., Savaris, C., Sise, H., Giourd, M., and McDermott, W. V., Jr. Extended preservation of the primate liver by simple cooling and orthotopic preservation. *Medical Primatology,* 1970. Proc. 2nd Conf. Exp. Med. Surg. Primates, New York, (Karger, Basel, 1971) pp. 119–129, 1969.
24. DeGasperis, M. A., and Donatelli, R. Ultrastructural changes in human myocardium associated with ischemic arrest. J. Mol. & Cel. Card. 1:109–174, 1970.
25. Denker, M. W., Berman, R. A., and Nachlas, M. M. Ultrastructural changes in myocardium during experimental ischemia. Johns Hopkins Med. J. 124:311–329, 1969.
26. Trunkey, D., Degenhardt, T., Chartrand, C., Pryor, J. P., and Belzer, F. O. Preservation of canine Hearts. Cryobiology 6:515, 1970.
27. Veith, F. J., Sinha, S. B. P., Graves, J. S., Boley, S. J., and Dougherty, J. S. Ischemic tolerance of the lung: Influence of ventilation and inflation. J. Thorac. Cardiovasc. Surg. 61:804, 1971.
28. Hankinson, H. W., and Edwards. The effect of pulmonary ischemia on lung function. Thorax 14: 122–124, 1959.
29. Sampson, D., and Murphy, G. P. Sequential changes in kidneys subjected to hypoperfusion. In: *Reversibility of Cellular Injury Due to Inadequate Perfusion.* C. H. Thomas, Springfield, (in press), 1971.
30. Tsuzuki, Y., Sakaguchi, H., Nakamura, Y. and Akakura, I. Keio J. Med. 15:111, 1966.
31. Garzon, A. A., Cheng, C., Pangan, J., and Karlson, K. E. Hypothermic hyperbaric lung preservation for twenty-four hours with reimplantation. J. Thoracic Cardiovascular Surg. 55: 546–554, 1968.
32. Collins, D. M., Bravo-Shugarman, M., and Terasaki, P. Kidney preservation for transplantation. Lancet 2:1219, Dec. 6, 1969.
33. Pegg, D. E., Calne, R. Y., Pryse-Davies, J., and Brown, F. L. Canine renal preservation using surface and perfusion cooling techniques. Ann. N.Y. Acad. Sci. 120:506–523, 1964.
34. Abbott, W. M., and Weinerth, J. L. Perfusion of isolated whole organs. Cryobiology 8:113–133, 1971.
35. Perry, V. P. Review of methods of organ perfusion and culture. In: *Microcirculation, Perfusion and Transplantation of Organs.* (Eds: T. I. Malinin, B. S. Linn, A. B. Callahan, and W. D. Warren), Academic Press, New York, 1970.
36. Belzer, F. O., Ashby, B. S., May, R. V., and Dumphy, J. E. Isolated perfusion of whole organs. In: *Organ Perfusion and Preservation.* (Ed: J. C. Norman), Appleton Century Crofts, New York, pp. 3–12, 1968.
37. Sinha, V. P., Atkinson, S. A., and Pierce, J. M., Jr. Comparative evaluation of two kidney preservation techniques in dogs. Lancet, pp. 421–423. Feb. 27, 1971.
38. Humphries, A. J., Jr., Russel, R., Stoddard, L. O., et al. Three-day kidney preservation: Perfusion with hypothermic, diluted blood or plasma. Surgery 63:646–652, 1968.
39. Nawrocki, C. Uber den Einfluss der Temperatur auf die Tatigkeit des Saugethierherzens. Inauguration Dissertation, Rostok, 1896.
40. Gollan, F. In vivo perfusion of hearts. In: *Microcirculation, Perfusion and Transplantation of Organs.* (Eds: T. I. Malinin, B. S. Linn, A. B. Callahan, and W. D. Warren), Academic Press, New York, 1970.
41. Feemster, J., and Lillehei, R. C. Preservation of kidneys using extracorporeal perfusion. In: *Microcirculation, Perfusion and Transplantation of Organs.* (Eds: T. I. Malinin, B. S. Linn, A. B. Callahan, and W. D. Warren), Academic Press, New York, 1970.

42. Belzer, F. O., Ashby, B. S., and Dunphy, J. E. 24-hour and 72-hour preservation of canine kidneys. Lancet 2:536–539, 1967.
43. Malinin, T. I. Processing and storage of viable human tissue. Public Health Service Pubc. No. 1442, U.S. Government Printing Office, Washington, D.C., pp. 45–54, 1966.
44. Malinin, T. I., and Perry, V. P. Toxicity of dimethyl sulfoxide on hela cells. Cryobiology 4:90–96, 1967.
45. Karow, A. M., Jr. Biological effects of cryopreservation as related to cardiac cryopreservation. Cryobiology 5:429–443, 1969.
46. Conway, H., Griffits, B. H., Shannon, J. E., and Findley, A. Survival of the transplanted fetal heart in the mouse as determined by pulsatile activity. Transpl. Bull. 5:121–123, 1958.
47. Rey, L. Physiologic du coer de l'embryon de poulet *in vitro* apres congelation a tres basse temperature. J. Emb. Exp. Morph. 6:171–177, 1958.

DISCUSSION

Chairman Frank J. Veith: I want to thank Dr. Malinin for his very nice presentation. I have one question. What are your thoughts about Belzer's recent observation that the kidney is unique in that it can be preserved by perfusion at 10°C because of its special enzyme system, and that the same sort of perfusion won't work for liver or other organs?

Dr. Malinin: I think this is quite correct, and it seems to be a difference between the kidney and heart, too. We obtain best results with the kidney at about 10°C, while the heart appears to survive better between 15 and 18°C. Perhaps we have not been successful in preserving liver because we simply haven't found the temperature at which this organ can be maintained *in vitro*.

Chairman: Your point that the PAH and inulin clearance on the perfusion pump are a good *in vitro* test shouldn't be dismissed lightly. If this is indeed the case, it is a very valuable contribution, because all of us who are faced with the problems of perfusing organs are also faced with the problem of putting in organs that don't work. Can you tell us how accurate this test is?

Dr. Malinin: Well, of course this work is preliminary. We have done it in rabbits only and have not extended it to people or larger animals. I would like to emphasize the difference between the clearance and the extraction. The clearance, if properly done by standard methods, is an extremely long and cumbersome procedure, and we need a test which will give results in 10–15 minutes. With extraction, we only need one sample taken concommitantly from the artery and the vein so we can measure the difference between the two. So, the data from extraction plus the pH give the necessary information. We have not had failures in reimplanted organs where extraction has been demonstrated, while we have had numerous failures with urine production and with adequate flow (which of course could be erroneous because of the ruptured blood vessels) in the organs which have been reimplanted without such testing.

Chairman: This is certainly a very exciting observation, and we will await your future comments on this particular test.

PANCREAS TRANSPLANTATION IN DOG AND MAN

F. K. Merkel*

Experience with the canine experimental model has shown that the pancreas can be transplanted and that such a graft can correct either surgically or medically induced diabetes. Operative techniques have been developed and the tolerable ischemic interval has been defined at one hour. Lessons learned in the laboratory have now been transposed to the clinical setting. The human pancreas transplantation experience is summarized.

INTRODUCTION

> "Though a simple operative curiosity today, the transplantation of a gland may perhaps some day have a practical application."
>
> Alexis Carrel, 1901

Animal models often cannot duplicate the human or his diseases. However, lessons learned from attempts at canine pancreas transplantation have been applied to the juvenile diabetic and served to encourage the use of the procedure in man.

Since the introduction of parenteral insulin for the treatment of juvenile diabetes mellitus by Banting and Best, prevention of ketoacidosis has been possible.[1] Despite careful use of insulin and diet, however, little has been done to prevent the progressive, destructive angiopathy of this disease.

Thus, the life span of an individual developing overt diabetes as a child is two-thirds that of the general population. The average age of death of a patient exhibiting diabetes mellitus by nine years of age is 35 years. Renal and cardiovascular problems cause 45 per cent of the deaths due to diabetes. Diabetes is also the third leading cause of blindness in the United States.[2]

This serious inherited metabolic defect afflicts more than 562,000 persons each year. Clearly, it is vital to find a better solution to this problem. Although numerous theories exist concerning the etiology of juvenile diabetes mellitus, pancreas allografting offers the only definitive means of testing the hypothesis that absence of a precise, moment-to-moment control of glucose metabolism results in the angiopathy. This angiopathy can be diffuse, affecting multiple organ systems, or may principally affect only one organ.

CANINE PANCREATIC TRANSPLANTATION

Pancreatic allografts were first successfully carried out in dogs by Gayet and Houssay using cannulas to effect the vascular anastomoses.[3,4] Clotting caused failure after several hours. A vascular suture technique was first used by Lichtenstein in 1957; however, venous thromboses resulted in a high incidence of failure.[5] Since that date, a variety of techniques were reported for whole organ transplants of the pancreas for its endocrine function. Essentially, such grafts consisted of either a segment of the pancreas, the pancreatico-duodenal complex, or the pancreas alone.[6-11] These studies demonstrated the technical feasibility of pancreas transplantation in the dog. Vascular complications were common in most of these preparations, however, causing failure of one-third to one-half of the grafts.

If the operation was to be tried in humans, it was essential to develop a more successful vascular technique for pancreas grafting. It appeared likely that the cause of these thromboses was the small blood supply the pancreas received in the dog; whereas a renal allograft receives one-quarter of the cardiac output, the pancreas only receives one to two per cent of the cardiac output. A technique was developed

* Northwestern University Medical School, Chicago, Illinois.

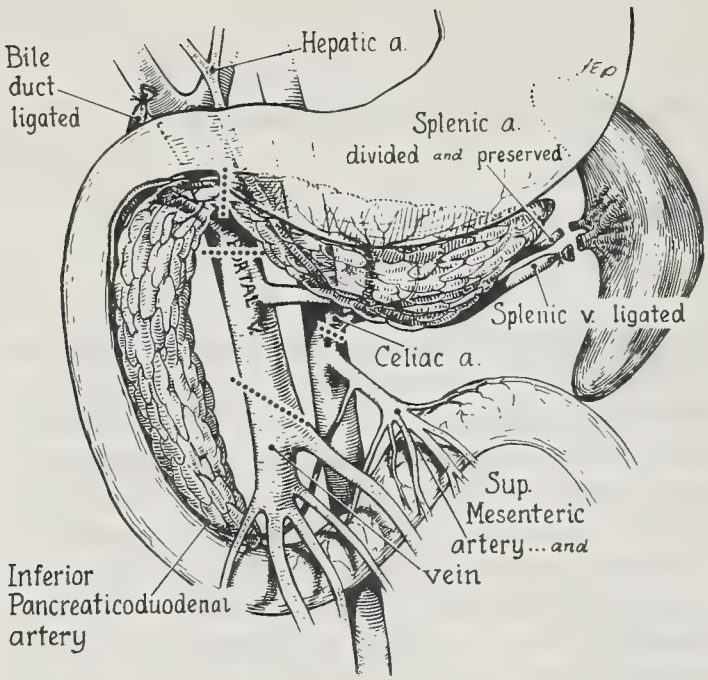


FIGURE 1.—The donor operation for segmental pancreatic allografting in the dog. The pancreas is ligated and divided at its neck. The portal vein-superior mesenteric vein comples is excised *en bloc* and the splenic artery is preserved for anastomosis. The arterial supply to the graft is the celiac artery. (Reprinted by permission of Dr. Merkel).

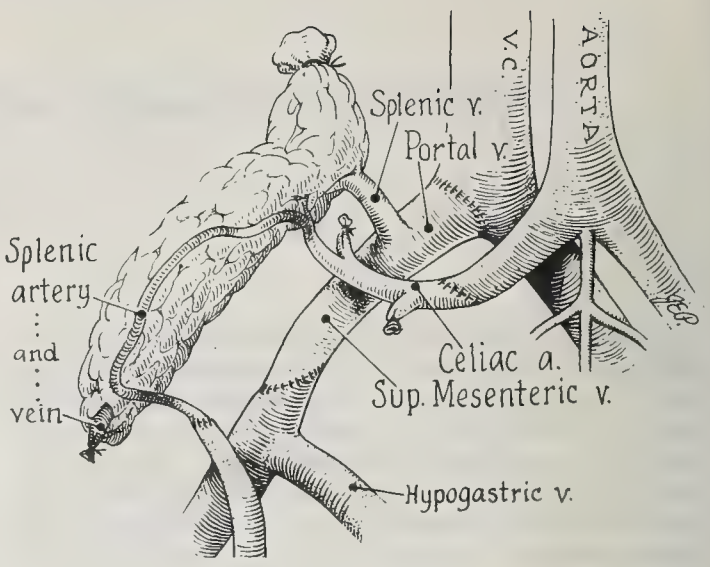


FIGURE 2.—The recipient operation for segmental pancreatic allografting in the dog. The portal vein of the graft is anastomosed to the proximal iliac artery at the vena cava and the superior mesenteric vein is connected distally to the iliac vein near the take off of the hypogastric vein. (Reprinted by permission of Dr. Merkel).

by Merkel interspersing the pancreas into the blood supply to and from the hind leg so that patency of the vascular connections was assured by the large hind limb flow[12,13] (Figures 1 and 2).

An early observation which was borne out in the human experience indicated the transplanted pancreas was most sensitive to manipulation and, following revascularization, points of trauma became hemorrhagic. Therefore, a "No Touch" technique was adopted for extirpation. The donor pancreas was ligated and divided at the neck and only the distal portion transplanted on a vascular pedicle consisting of the portal and superior mesenteric veins and the celiac and splenic arteries.

In the previously pancreatectomized or alloxanated recipient, both the iliac artery and vein were divided between vascular clamps. Anastomoses were carried out between the donor portal vein and recipient vena cava, the donor superior mesenteric vein and the recipient distal iliac vein, the donor celiac axis and the host's proximal iliac artery, and the recipient's distal iliac artery with the donor splenic artery (Figure 3). This technique was remarkably effective and abolished thrombosis as a cause of failure. These studies also showed that either conventional immunosuppression with Imuran and prednisone, or antilymphocyte serum could result in prolonged graft survival (Table I). Survival of up to 98 days was obtained with this method (Figure 4).

The anatomic and histopathologic consequences of pancreatic allografting were similar to those encountered in renal allotransplantation. The gross appearance of the rejecting pancreas is much like the rejecting kidney (Figures 5 and 6). The gland is edematous and

TABLE I

Group	Survival	Vascular Thrombosis	Leakage of Pancratic Secretions	State of Graft
A. Pancreatectomized Dogs (15 Dogs)	15.6d. (9-55d.)	0	2	53 % Functioning at Death
B. Alloxinated Dogs (15 Dogs)	31.0d. (2-98d.)	0	0	40 % Functioning at Death

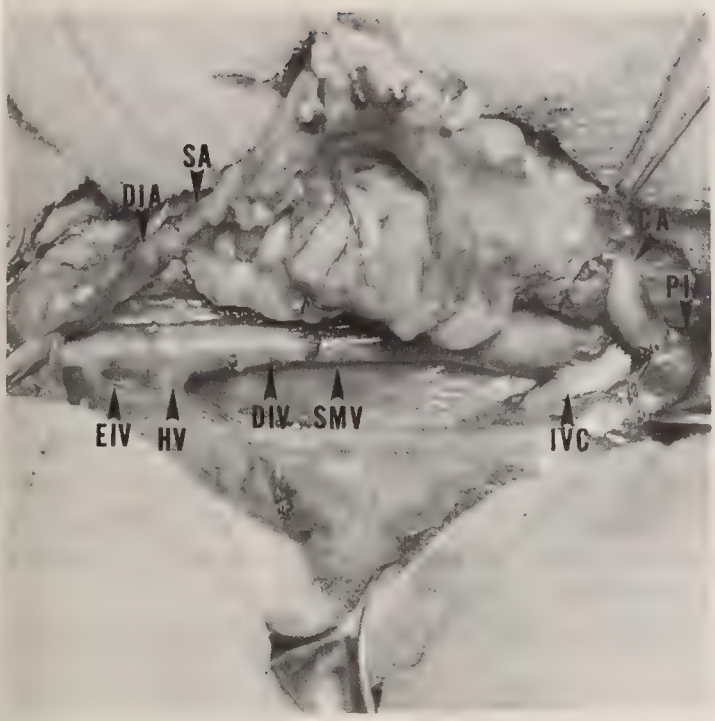


FIGURE 3.—The canine segmental pancreatic allograft following completion of the anastomoses. The celiac axis (CA) is anastomosed to the proximal iliac artery (PIA) and the splenic artery (SA) to the distal iliac artery (DIA). The portal vein (PV) is sutured to the inferior vena cava (IVC) at the take off of the iliac vein and the superior mesenteric vein is sutured to the distal iliac vein (IV) proximal to the junction of the external iliac vein (EIV) and the hypogastric vein (HV). Note the delicate appearance of the transplanted gland and the freedom from hemorrhage.

hemorrhagic with a markedly thickened capsule. A marked round cell infiltrate is seen on histologic section (Figure 7). The non-rejecting canine transplant invariably exhibits prominent beta cell granulation to the aldehydefuchsin reaction. The transplanted gland responds to the prednisone used for immunosuppression by sprouting off new beta cells from the ductile system of the gland in just the same way as a normal, non-transplanted pancreas responds to steroids (Figure 8). Some of the duck ligated glands which served more than six weeks exhibited marked fibrosis (Figure 9).

Studies were carried out to determine the effect of ischemia on the pancreas transplant in the laboratories of Largiader, Bergan and Merkel (14–17). A significant finding was that the fasting blood sugar was not the best indicator of the presence or absence of ischemic injury (Figure 10). For, although normal fasting blood sugar was recorded after 90 minutes of normothermic ischemia, glucose tolerance curves clearly demonstrated the damage incurred by the transplant. At 90 minutes, the damage appeared reversible; however, at 190 minutes, the glucose tolerance and insulin curves were permanently altered (Figure 11). Ischemia also resulted in early hyperinsulinemia, presumably a result of injury to the beta cell membrane causing increased permeability. Thus, one hour was predicted as the maximum period of normothermic ischemia allowable in a human pancreatic transplant.

The canine experiments thus demonstrated the technical feasibility of pancreatic trans-



FIGURE 4.—Alloxinated dog following pancreatic allografting several weeks prior to sacrifice at 98 days. Note his healthy appearance. He exhibited normal blood glucose and insulin levels in the absence of glycosuria. Glucose tolerance curves were within normal limits.

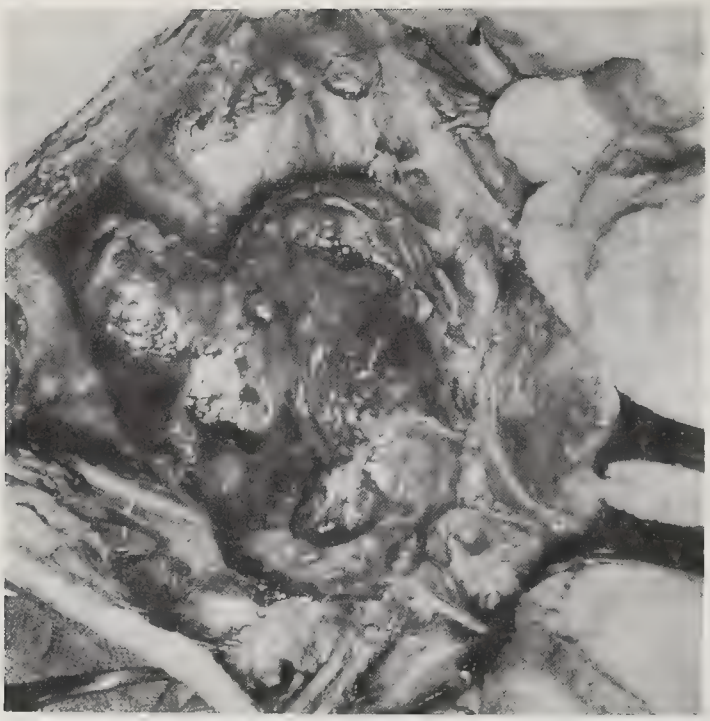


FIGURE 5.—The rejecting canine pancreatic allograft. Note the edema and hemorrhage characteristic of far advanced rejection.

plants and established their response to ischemia and to conventional systemic immunotherapy.

HUMAN PANCREATIC TRANSPLANTATION

The first attempt at a vascularized transplant of the pancreas for end stage diabetic nephropathy in the human was carried out by Kelly, Lillehei, Merkel, Idezuki and Goetz at the University of Minnesota on December 17, 1966.[18,19] The same principles which had proven successful in the dog were employed to assure patency of the venous connections (Figure 12). The graft consisted of the body and tail of the pancreas distal to the portal vein. The duct was ligated and the raw edges were oversewn. The configuration of the human pancreas did not allow for similar treatment of the arterial supply. Hence, the celiac axis alone was anastomosed end-to-end to the iliac artery. The transplanted pancreas was placed in the left iliac fossa and a kidney was transplanted from the same donor to the right iliac fossa. Renal function was never achieved and, although control of the blood sugar was achieved, it was evanescent. After four or five days, the endocrine function of the pancreas ceased. Interestingly, the exocrine function of the graft persisted for four and one-half months.

Because of leakage of exocrine secretions from the ligated duct, the next patient received a pancreatico-duodenal graft. The proximal end of the duodenum was oversewn and the distal end was fashioned into a mature stoma in the left lower quadrant of the abdomen. It was possible to observe and biopsy the stomal mucosa and collect secretions for analysis. Both renal and pancreatic grafts functioned satisfactorily in this patient; however, she died of pulmonary sepsis four and one-half months following transplant.

This patient interestingly exhibited a rejec-



FIGURE 6.—The rejecting canine kidney. Compare the features with that of the pancreas. There is a much thicker capsule on the kidney; however, the organ is swollen and edematous with beginning hemorrhages.

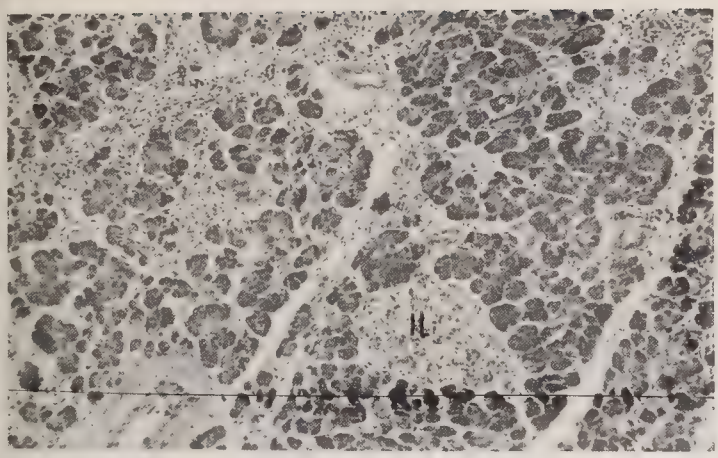


FIGURE 7.—The histopathology of the rejecting canine allograft. This is acute rejection characterized by a round cell invasion of the gland including the islets of Langerhans (IL), widening of the interlobular fissures and disruption of the acinar tissue. These features are common to renal transplantation and indicate that endocrine tissue is not immune from immunologic attack.

tion reaction two weeks after the operation. This was directed against the pancreas, the duodenum and the kidney, and was characterized by oliguria, elevated blood urea nitrogen and creatinine, elevated blood sugar, fall in circulatory plasma insulin, and a decrease in the amylase content and volume of the duodenal juice.

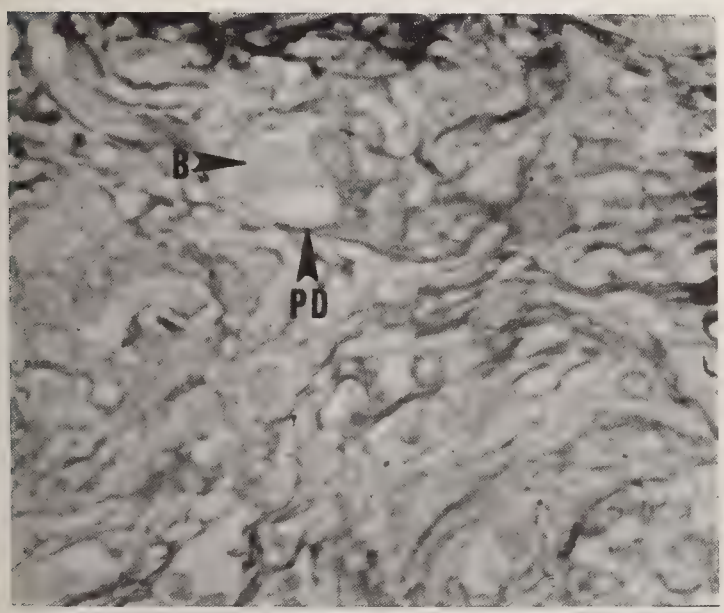


FIGURE 8.—Budding off of new beta cells in the transplanted canine pancreas. This is occurring under the influence of steroids used to prevent rejection. Note the beta cells (B) arise from the epithelium of the pancreatic duct (PD).

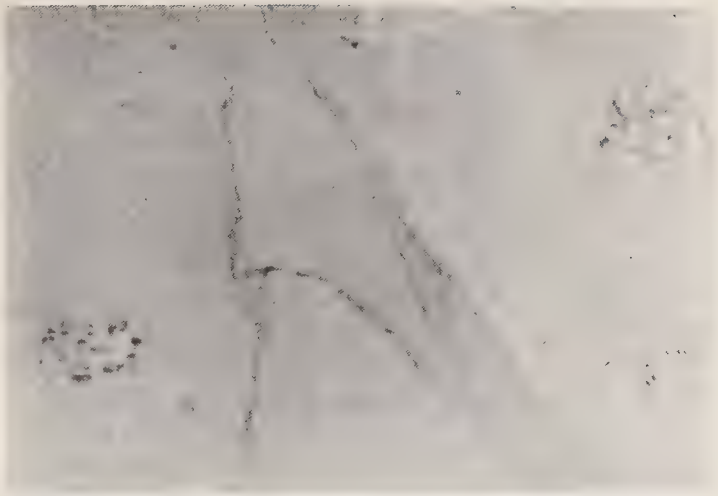


FIGURE 9.—Fibrosis appearing in biopsy taken 55 days after transplantation of this canine pancreas.

The duodenal mucosa became grossly edematous, hemorrhagic, and sloughed. A massive hemorrhage from the stoma occurred requiring transfusion and iced saline lavage of the duodenal segment. Biopsies showed a round cell infiltrate and mucosal slough of the duodenal segment. With appropriate anti-rejection therapy, both renal and pancreatic function improved and the mucosa of the stoma regenerated. The

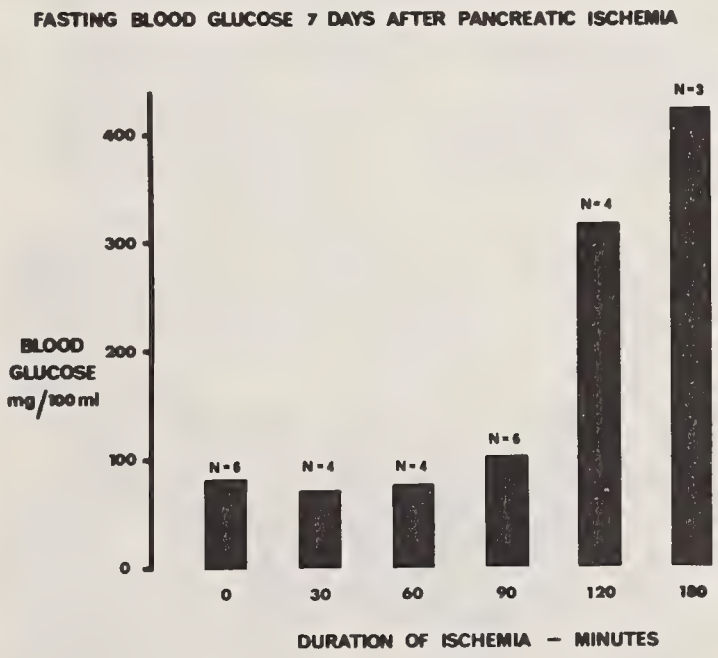


FIGURE 10.—Fasting blood sugar levels after varying periods of normothermic ischemia to the isolated pancreas segment in the dog. Note that no abnormality is seen until 120 minutes.

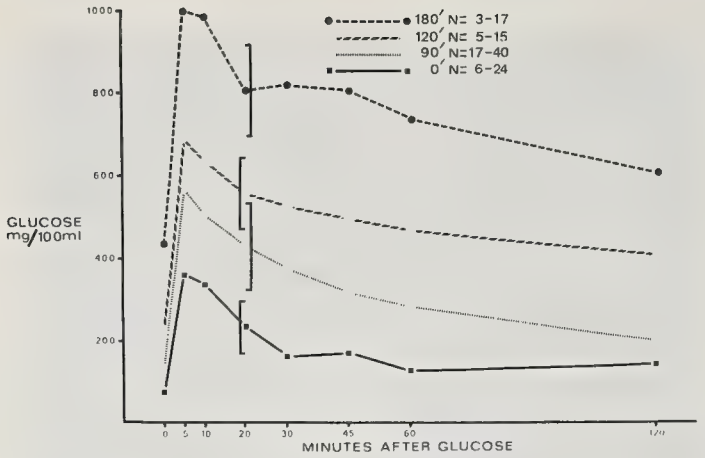


FIGURE 11.—Glucose tolerance curves demonstrate greater sensitivity to the effects of normothermic ischemia on the canine pancreas.

present technique favored by the author is illustrated in Figure 13.

To date, a total of twenty-four transplants of the pancreas have been carried out in man at nine centers (Table II). The grafts have been both segmental and pancreatico-duodenal, with and without an associated renal allograft. Maximal survival has been twelve months. Those surviving six months or more with functioning

TABLE II.—*Pancreas Transplantation in Man*

Type of Transplant	Number of Cases	Graft Survival (Range)
Pancreas (Body and Tail)	5	6 Days—120 Days
Pancreas/Kidney	2	59 Days—3 Months +
Pancreatico-Duodenal	3	7 Days—2 Months
Pancreatico-Duodenal/Kidney	14	5 Days—12 Months

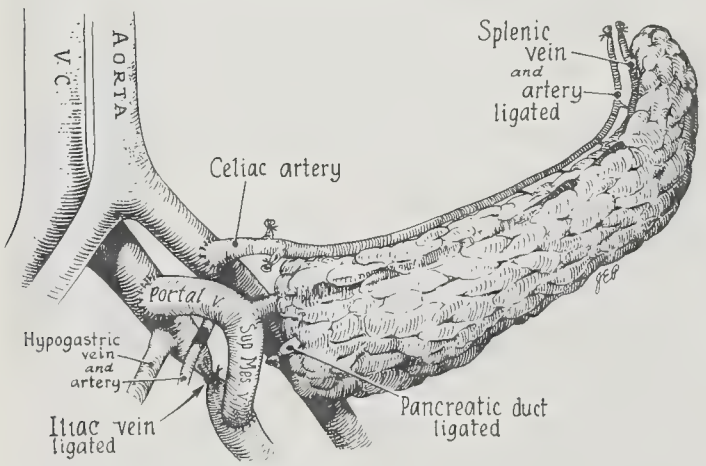


FIGURE 12.—Technique employed in the first human pancreatic allograft, December 17, 1966. Note the vascular connections and the ligated pancreatic duct.

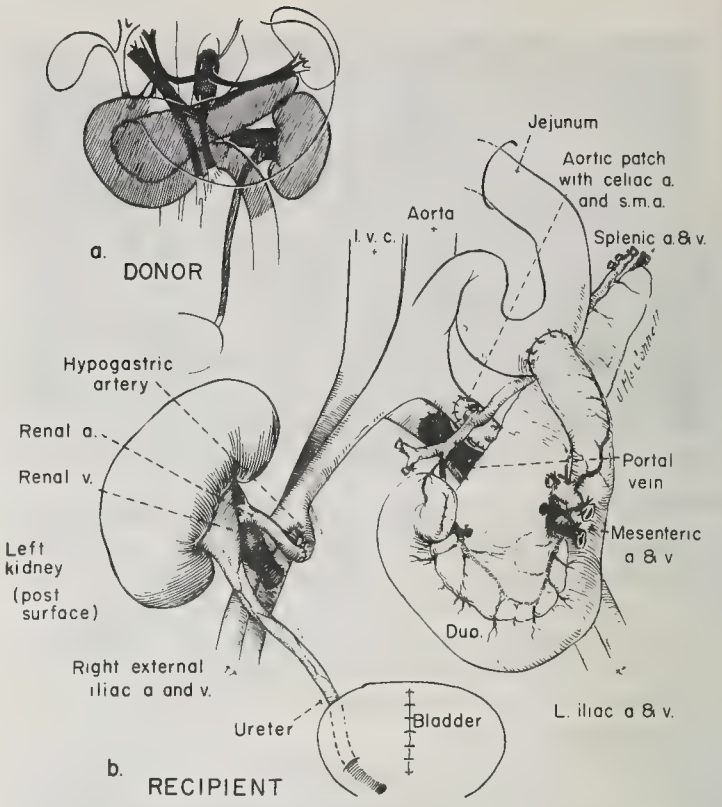


FIGURE 13.—Technique for pancreatico-duodenal transplantation in man for juvenile diabetes with renal failure. Note the *en bloc* extirpation of the gland. The renal transplant is depicted using the technique usually employed. The pancreas is transplanted with a Carrel patch of aorta containing the origins of the celiac and superior mesenteric arteries.

grafts have clearly demonstrated that excellent control of sugar metabolism could be obtained. None have survived long enough to determine the effect on angiopathy. However, none of the longer survivors exhibited worsening of their angiopathy, especially with respect to retinal vasculature.

The clinical experience has underlined some of the principles learned from the canine experiments. The "no touch" technique has proven to be indispensable to removing a gland which will continue to function normally in the recipient.[20] Immediate post-transplant hyperinsulinemia has been seen in the human as well as the dog. The only present survivor of pancreas transplantation in man received a pancreas without duodenum followed by a renal allograft at a second operation. Doctors Veith and Gliedman employed an ingenious device to provide for pancreatic duct drainage. They anasto-

mosed the pancreatic duct to the nearby ureter. A report of this fascinating case is anticipated in the near future.

SUMMARY

Human pancreatic transplantation is in its embryonic stage and yet, should success be achieved, an unending supply of eager recipients would be available. The question of whether or not the transplanted gland would control glucose metabolism in the human has been answered in the affirmative. Only the lack of adequate financial support has prevented the more widespread trial of such grafts to determine the effect on the angiopathy of this disease. Such trials must and will be done to establish whether pancreas transplants will achieve the same clinical usefulness as renal transplants have in man.

REFERENCES

1. Banting, F. G., Best, C. H., Collip, J. B., Campbell, W. R., and Fletcher, A. A. Pancreatic extracts in the treatment of diabetes mellitus. Canad. Med. Assn. J. 12:141, 1922.
2. *Diabetes Source Book.* Washington, D.C., U.S. Department of Health, Education and Welfare, 1968.
3. Gayet, R., and Guillaumie, M. La regulation de la secretion interne pancreatique par un processus humorale, demontree par des transplantations du pancreas. Experiences sur des animaux normaux. C R Soc Biol (Paris) 97:1613, 1927.
4. Houssay, B. A., Lewis, J. T., and Foglia, V. G. Action compensatrice ou preventive de la greffe pancreatique sur la glycemie diabetique ou normale. C R Soc Biol (Paris) 100:140, 1929.
5. Lichtenstein, I. L., and Barschak, R. N. Experimental transplantation of the pancreas in dogs. J. Int. Coll. Surg. 28:1, 1957.
6. Lucas, J. F., Jr., Rogers, R. E., and Reemtsma, K. Homologous pancreatic transplantation in dogs: Report of a new technique in studies of endocrine function. Surg. Forum 13:314, 1962.
7. Reemtsma, K., Lucas, J. F., Jr., Rogers, R. E., Schmidt, F. E., and Davis, F. J., Jr. Islet cell function of the transplanted canine pancreas. Ann. Surg. 158:648, 1963.
8. Largiader, F., Lyons, G. W., Hidalgo, F., Dietzman, R. H., and Lillehei, R. C. Orthotopic allotransplantation of the pancreas. Amer. J. Surg. 113:70, 1967.
9. DeJode, L. R., and Howard, J. M. Studies in pancreatico-duodenal homotransplantation. Surg. Gynec. Obstet. 114:553, 1962.
10. Teixeira, E., Sharkey, E., Colwell, J., and Bergan, J. J. Insulin and glucose levels following pancreas allografting. Surg. Forum 17:205, 1966.
11. Bergan, J. J., Hoehn, J. G., Porter, N., and Dry, L. Total pancreatic allografts in pancreatectomized dogs. Arch. Surg. 90:521, 1965.
12. Merkel, F. K., Kelly, W. D., Goetz, F. C., and Maney, J. W. Heterotopic dog pancreatic allografts. Surg. Forum 18:381, 1967.
13. Merkel, F. K., Kelly, W. D., Goetz, F. C., and Maney, J. Irradiated heterotopic segmental canine pancreatic allografts. Surgery 63:291, 1968.
14. Moser, R., Meili, H. U., and Largiader, F. Die Ischamietoleranz des Pankreas. Z Ges. Exp. Med. 143:266, 1967.
15. Binder, P. S., Heiskell, C. A., Adams, J. R., Cerra, F. B., Eilert, J. B. and Bergan, J. J. Pancreatic function after normothermic ischemia. I. *In vivo* ischemia. Amer. J. Surg. 120:687, 1970.
16. Cerra, F. B., Adams, J. R., Eggert, D. E., Eilert, J. B., and Bergan, J. J. Pancreatic function after normothermic ischemia: II. Cadaveric transplantation. Amer. J. Surg. 120: 693, 1970.
17. Douglas, M., Richard, B., Eiseman, B., and Merkel, F. K. The effect of ischemia on the endocrine function of the canine pancreas (Abstract). Presented at the 4th World Congress of Gastroenterology, Copenhagen, July, 1970.
18. Kelly, W. D., Lillehei, R. C., Merkel, F. K., Idezuki, Y., and Goetz, F. C. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery 61:827, 1967.
19. Kelly, W. D., Lillehei, R. C., Merkel, F. K., Idezuki, Y., and Goetz, F. C. Renal and pancreatic allotransplantation in the treatment of diabetic nephropathy in man. In *Advance in Transplantation,* Dausset, J. et al. (Eds.), Baltimore, Williams and Wilkins, 1968.
20. Merkel, F. K., Poticha, S. M., Nudelman, E. J., Colwell, J. A., and Bergan, J. J. Pancreas transplantation in man: I. The donor and recipient operation. Arch. Surg. 103:205, 1971.

DISCUSSION

Chairman Frank J. Veith: Thank you Dr. Merkel. I'd like to give you some details about a patient of ours with renal disease and severe diabetes. Because many problems have developed with the conjunction of duodenal and kidney transplants, we elected to treat this woman by transplanting just the tail of her pancreas and anastomoting her pancreatic duct to her ureter. This sounds like a bizarre thing to do, but we had already tried the anastomosis of the pan-

creatic exocrine duct to the ureter in five dogs, all of whom seemed to tolerate this procedure well. For a period of 5–7 months, these dogs have manifested no urinary tract or pancreatic difficulties. So on the strength of that experience, we did the same form of anastomosis on our patient. She has done reasonably well; her blood sugars have remained normal, her insulin requirement has dropped from 50–70 units a day to zero, and her blood sugar has been persistently normal. A month or two following transplantation she developed a small leak from the ureter to the pancreatic duct, but this is essentially closed to the point where she now puts out between zero and 10 cc. of clean pancreatic juice per day. The rest of it comes out her bladder. About 3 weeks ago she received a kidney transplant and her own two kidneys were removed, and she is doing quite well and is ready for discharge.

ACKNOWLEDGMENTS

Supported in part by grants from the American Diabetes Association, the Colorado Heart Association, the Twin Cities Diabetes Association, the Minnesota Heart Association, and the Northwestern University Transplantation Division.

LEFT VENTRICULAR ASSIST DEVICES IN THE CALF: ANESTHESIA, VENTILATION, ANTIARRHYTHMIC REGIMEN, AND IMPLANTATION

Farouk A. Molokhia, Fred N. Huffman, William J. Robinson, Panayiotis J. Asimacopoulos, Ronald B. Ponn and John C. Norman*

Our initial procedures for LVAD implantation in the calf have been complicated by (1) inadequate pulmonary function during anesthesia, (2) cardiac dysrhythmias, (3) excessive blood loss and (4) coronary air embolism. During our subsequent experience a series of adjuncts was evolved to circumvent these problems. Calves weighing 91±3.6 kg were premedicated with 0.4—0.8 mg of atropine sulphate. Anesthesia was induced with 20–30 cc of 2.5% sodium thiamylal. Intubation was often difficult and muscle relaxants were avoided until after assisted ventilation had been instituted. Anesthesia was maintained at a light plane by inhalation of 0.5–1.0% Halothane. The animals were ventilated with a pressure-cycled respirator delivering 50% oxygen at 15 breaths/minute. In the first series of calves ventilatory pressures (VP) were set at 15–20 cm H_2O, resulting in tidal volumes of 1000–2000 ml. In the second group of calves VP's were set at 30–40 cm H_2O, with tidal volumes of 1400–1600 ml. Only in the second group was arterial pO_2 maintained at adequate levels. The results 150 minutes following induction of anesthesia were:

	pH	pCO_2(mmHg)	pO_2(mmHg)
Low VP	7.45±0.04	42.1±6.9	77.5±7.1
High VP	7.45±0.08	28.9±2.5	228.0±28

These results indicated that high VP's result in significant ($p<0.01$) increases in tidal volumes and arterial pO_2's and acceptable decreases in pCO_2's. The antiarrhythmic precautions employed included preoperative correction of potassium deficiencies, the use of lidocaine at the beginning of the procedure (80 mgIV) as a continuous infusion (1 mg/min) and intraatrially (30 mg) before intracardiac manipulation, and administration of 100% O_2 during intracardiac manipulation. Ventricular fibrillation did not occur in animals receiving this regimen. The implantation technique employed was based on that described by Bernhard with modifications. The use of a single balloon catheter simplified the original method and was combined with CO_2 flushing of the pump through the inflow tube and CO_2 flooding of the operative field. These measures have markedly decreased blood loss and eliminated coronary air embolism. In our experience attention to these details and employment of these adjuncts have greatly simplified the procedure and should be useful to others.

* From the Department of Surgery, Harvard Medical School, the Cardiovascular Division, Sears Surgical Research Laboratories, Harvard Unit, Boston City Hospital, Boston, Massachusetts, and the Thermo Electron Corporation, Waltham, Massachusetts.

** Supported, in part, by USPHS Contracts: PH-NHLI-43-67-1116 and PH-NHLI-43-68-1455.

INTRODUCTION**

The calf is being utilized with increasing frequency as an animal model for the development of circulatory support systems. The size, anatomic relationships of thoracic structures, major parameters of circulatory hemodynamics and the blood coagulation mechanisms make this animal especially useful in the development of devices for possible use in humans. However, investigators using calves for the development and evaluation of complex cardiac devices have been hampered by the development of pulmonary insufficiency and the occurrence of cardiac dysrhythmias intraoperatively. In the specific area of left ventricular assist pump implantation, additional problems have been excessive blood loss and coronary air embolism. The purpose of this paper is to summarize our recent experience with left ventricular assist device (LVAD) implantation in twenty-four calves weighing between 80 and 102 kilograms. The basic technique employed is that developed by Bernhard,[1,2] with modifications in anesthesia, ventilation, antiarrhythmic precautions and methods of LVAD implantation and carbon dioxide flushing.

ANESTHESIA AND VENTILATION

A major difficulty encountered in our initial procedures was severe, uncorrectable hypoxia and moderate hypercarbia. This problem was approached by (1) general measures designed to identify and eliminate from the series animals with preexisting pulmonary disease, (2) careful selection of an appropriate anesthetic regimen and (3) definition and correction of intraoperative mechanical factors that might result in inadequate ventilation.

In order to insure that only calves in good health were utilized, the physical status of all animals was evaluated preoperatively. Those with poor appetities, diarrhea or rectal temperatures above 102° F were eliminated from the study.[3] The chests were auscultated for evidence of pneumonia, a common disease in commercially obtained young calves,[4] and white blood cell counts and differentials were determined. (Normal range: 6,000–12,000/mm^3.)

Despite these measures, however, several of the animals undergoing thoracotomy showed gross evidence of acute pneumonia and almost all showed evidence of chronic pulmonary fibrosis, mainly involving the lower lobes and lingula. Accordingly, antibiotic treatment with Ampicillin (Wyeth Laboratories, Inc., Philadelphia, Pa.) (1 gm/day). The effectiveness of such treatment, however, is questionable, since pulmonary consolidation persists long after acute infection subsides.[5]

Bacteriological examination of exudate from calves that developed empyema postoperatively showed the presence of *Pasteurella multocida*, a small, non-spore-forming, gram-negative bacillus, particularly common in the respiratory tracts of animals.[6,7] The strain in our laboratory is sensitive to ampicillin, penicillin, tetracycline and erythromycin, but resistant to lincomycin, streptomycin and nafcillin.

Profuse salivary and tracheobronchial secretions are characteristic of ruminants.[3] These were partially controlled by intramuscular injection of 0.4–0.8 mg of atropine sulphate (Eli Lily and Company, Indianapolis, Indiana) prior to induction of anesthesia. Although scopolamine has also been used effectively,[8] it was not used in these experiments since idiosyncratic reactions to therapeutic doses in humans are more common than with atropine. The same doses that normally produce drowsiness may instead cause excitement and delirium.[9]

Food is withheld the day before operation but free access to water is allowed since the stomachs of ruminants cannot be emptied by withholding water for 12 hours.

Anesthesia was induced by intravenous injection of 40–50 cc of 2.5% sodium thiamylal (Surital®, Parke-Davis, Detroit, Michigan) via a large intracath inserted in the left saphenous vein. This was administered slowly until the corneal reflex could no longer be elicited. Although others have reported that calves are extremely sensitive to even the ultrashort-acting barbiturates[10,11] and induce anesthesia by inhalation,[3,5,12,13,14] our experience with intravenous sodium triamylal for induction has been satisfactory. This might be due to the very light plane of anesthesia induced. In addition, the use of an intravenous barbiturate to produce loss of consciousness prior to initiation of inhalation anesthesia may offer the advantage of avoiding the undesirable stage of excitement more regularly seen with inhalation anesthetics.[3,9] Thiopental[2] and methohexital[8] have also been used for induction of anesthesia with apparent success.

The calves were intubated with 20 mm cuffed endotracheal tubes, 50 cm long (Rusch, West Germany) with manual abduction of the mandible and maxilla and a standard adult laryngoscope blade (The Foregger Co., Long Island, New York) extended 15 cm by attachment of a flat metal bar to a total length of 34 cm. Although muscle relaxants have been used for intubation,[15] they were not employed in this study to avoid paralysis of the respiratory muscles and irreversible hypoxia before assisted ventilation could be begun. However, prior to thoracotomy each animal received 80–100 mg of gallamine triethiodide (Flaxedil®, Davis and Geck, Danbury, Connecticut) intravenously for muscular relaxation. This compound has less sympathetic ganglionic blocking action than most neuromuscular blocking agents, but in effective doses does block parasympathetics.[9] These actions are not undesirable since they may help to avoid the potential bradycardia and hypotension caused by Halothane.

Anesthesia was maintained with 0.5–1.0% Halothane (Ayerst Laboratories, New York, New York) (2-bromo-2-chloro-1:1:1-trifluoroethane) delivered by a Fluotec vaporizer (Fraser Sweatman, Inc., Buffalo, New York). Halothane is the anesthetic agent most commonly employed for cardiovascular surgery in calves[1,2,5,10,12,14,15] although ether[3] and methoxyflurane[8] have also been used, but the former is flammable and precludes the use of electrocautery, while animals anesthetised with the

latter have been reported to require two hours postoperatively before resumption of spontaneous breathing.[16] Halothane was preferred to other inhalation anesthetics since it is nonflammable, causes only mild respiratory depression, and in the concentrations used produces little cardiac and circulatory depression. Although mixtures of nitrous oxide and a more potent anesthetic have been used in the calf,[2,13] N_2O was not employed during our procedures since the high concentrations required would restrict the fraction of oxygen available in the inspired gas mixture. In fact, although clinical observations suggest a better postoperative course, there is no definite evidence that nitrous oxide plus a more potent anesthetic is preferable to the volatile agent alone.[9]

Ventilation was the largest problem. Traditional methods of estimating tidal volumes by observing chest wall excursion during inspiration are unreliable. The calf's chest wall is extremely thick and inelastic. During spontaneous respiration, the chest wall exhibits little motion with diaphragmatic movement predominating.

All animals were ventilated with a Bennett (Bennett Respirator Products, Santa Monica, California) PR–1 pressure-cycled respirator with attached spirometer, delivering 50% oxygen at a rate of 15 breaths/minute. In an initial series of eight calves the respirator was set at inspiratory pressures of 15–20 cm H_2O. Tidal volumes ranged between 1000 and 1200 ml initially. Animals in this group developed hypoxia, moderate hypercarbia and acidosis soon after thoracotomy, and the tidal volumes dropped to 600–800 ml. Large amounts of sodium bicarbonate were necessary to maintain pH. Arterial pO_2 fell as low as 75 mmHg during assisted ventilation intraoperatively and 70 mmHg during spontaneous oxygen breathing postoperatively. Corresponding values of O_2 saturation and pCO_2 were 93% and 92%, and 42 and 48 mmHg, respectively. These values may be compared to normal pressures and saturation at 37° C and at body temperature determined by Donawick[17] in calves of comparable size (Table I). A more detailed representation is given in Table II and graphically in Figure 1. The abnormal blood gases encountered at low ventilatory pressures were not correctable by manual deep inflation of the lungs at 10–15 minute intervals. Intermittent use of 100% oxygen inhalation was also ineffective in producing sustained increases of pO_2 to acceptable levels.

In a second series of eight calves inspiratory pressures were raised to 30–40 cm H_2O. Tidal volumes were 1400–1600 ml initially. In contrast to the earlier group, pO_2 was maintained above 150 mmHg and tidal volume above 1200 ml during the entire procedure, with O_2 saturations above 96%. pCO_2 decreased to a minimum of 29 mmHg but generally remained within acceptable limits (Table II, Figure 1). These results indicate that ventilatory pressures of

TABLE I.—*Values for pH and blood gases in normal calves (from Donawick et al.[14])*

	Minimum	Maximum	Mean ± S. D.
pH			
37°C	7.37	7.46	7.40 ± 0.02
Body temp.	7.37	7.44	7.37 ± 0.02
Arterial pCO_2 (mmHg)			
37°C	34.7	44.0	39.5 ± 3.06
Body temp.	36.9	48.5	42.8 ± 3.28
Arterial pO_2 (mmHg).			
37°C	72.5	94.5	83.9 ± 83.9±6.30
Body temp.	79.0	108.6	93.6 ± 7.68

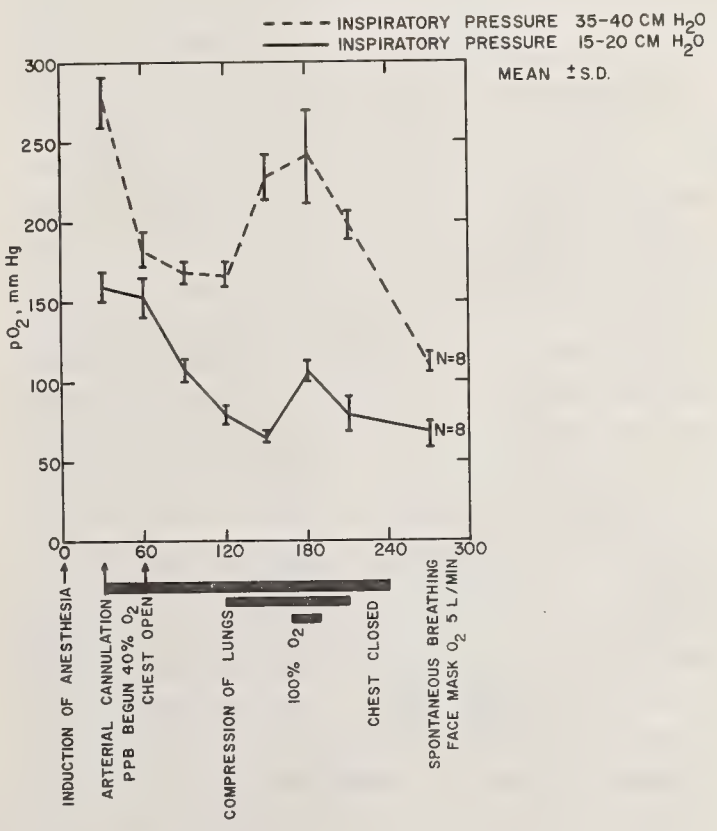


FIGURE 1.—Arterial pO_2 levels in calves during LVAD implantation under different ventilatory pressures.

TABLE II.—*pH, Blood Gases and Oxygen Saturations (Mean ± SD) During LVAD Pump Implantation in Calves Using IPBB (Two Ventilatory Pressures)*

	surgical manpulation								
	arterial cannulation		chest open		left of lung retraction of		chest closure	spontaneous breathing	
	IPBB							Face Mask + O_2 5 L/min	
	40%					100%			
	7.50	7.54	7.48	7.45	7.43	7.39	7.4	7.39	N = 8
pH	±0.03	±0.03	±0.01	±0.04	±0.07	±0.01	±0.02	±0.01	
pCO_2, mm Hg	35.1	32.5	35.0	38.6	42.1	40.4	39.4	48.2	
	±1.2	±1.7	±5.5	±5.0	±6.9	±3.4	±3.7	±5.0	Ventilatory Pressure 15–20 cm H_2O
pO_2, mm Hg	164.	154	112.0	80.5	77.5	112.4	82.5	69.4	
	±22	±26	±13.0	±12.0	±7.1	±15.7	±18.0	±12.0	
O_2	97.5	97.3	94.8	94.1	93.5	96.3	90.5	92.8	
	±0.3	±0.2	±2.4	±1.4	±0.5	±0.2	±2.5	±4.0	
	7.49	7.44	7.45	7.42	1.45	7.42	7.32	7.48	N = 5
pH	±0.02	±0.09	±0.03	±0.05	±0.08	±0.07	±0.07	±0.03	
pCO_2, mm Hg	24.9	37	32.5	30.7	28.9	30.7	32.8	35.9	
	±0.8	±3.9	±4.3	±4.1	±2.5	±4.1	±3.8	±2.0	Ventilatory Pressure 30–40 cm H_2O
pO_2, mm Hg	271	181	170.3	167	228	238	203	113.9	
	±42	±26	±6.6	±28	±28	±28	±13	±9.0	
O_2	97.6	97	96.6	96.8	96.7	97	97	96.5	
	±0.3	±0.3	±0.5	±0.3	±0.2	±0.2	±0.2		

30–40 cmH_2O resulted in significant ($P < 0.01$) increases in tidal volume and pO_2.

Conflicting reports have appeared with regard to optimal ventilatory pressures in the calf. Larson et al.,[10] working with young calves weighing less than 15 kg reported that pressures about 10 cm H_2O caused rupture of alveoli and extensive pulmonary hemorrhage. With these possibilities in mind, several investigators have used lower pressures than those employed in our second group of animals[18] in calves weighing between 61 and 110 kg. Donawick et al. found that pressures above 30 cm H_2O were necessary to prevent atelectasis and maintain adequate oxygen pressures during sham thoracotomies, while pressures below 20 cm H_2O resulted in hypoxia, hypercapnia, and atelectasis.[12] Our experience with high inspiratory pressures has been similar. The efficacy of high pressures might be explained by the findings of McLaughlin and associates[19] who reported on the well-developed lobularity of the calf lung, the thick inelastic pleura, the poorly developed respiratory bronchioles, and the presence of bronchopulmonary anastomoses.

An additional mechanical factor necessitating higher inspiratory pressures during left ventricular assist device implantation is the increase in abdominal pressure produced in left lateral position. This problem is frequently compounded in the calf by abdominal distention caused by bacterial fermentation and gas accumulation in the rumen during anesthesia. The normal calf produces 60 to 80 liters of CO_2 and methane per day[20] which accounts for the marked abdominal distention occasionally seen within a few hours after thoracotomy. Contributory factors include loss of eructation under general anesthesia and the poorly understood bloating syndrome. Withholding of food for 24 hours prior to surgery is not always effective in preventing abdominal bloating. Large caliber nasogastric tubes were used and proved to be of some value. On occasion, however, more complete decompression was necessary and was achieved by insertion of a 14 gauge 3″ needle into the rumen through the left flank. Recently, temporary gastrostomy has been found to be more efficient for gastric decompression.

An additional mechanical measure that can be utilized consists of increasing the resistance to expiration at intervals in order to increase functional residual volume and thus prevent progressive collapse of alveoli and decrease any degree of shunting.[21]

ANTIARRHYTHMIC REGIMEN

The sudden occurrence of cardiac dysrhythmias and ventricular fibrillation is another ma-

jor problem encountered in left ventricular assist device implantation and in all procedures in the calf entailing manipulation of the heart. Defibrillation is extremely difficult and has been performed successfully in only a few instances. It is therefore of paramount importance to prevent the development of ventricular dysrhythmias.

In the first group of eight animals ventilated at pressures of 15–20 cm H_2O, no specific antiarrhythmic measures were undertaken, and fibrillation occurred during implantation in all animals. In only one case was defibrillation successful with multiple 400 joule DC countershocks.

The first animal in the series ventilated at pressures of 30–40 cm H_2O also received no specific antiarrhythmic treatment. Despite the facts that the introduction of higher ventilatory pressures minimized abnormalities of blood gases and acid-base balance and that prompt correction of any disturbances was possible, fibrillation occurred in this animal as well. Thus the elimination of hypoxia and acidosis was not sufficient to prevent fibrillation.

Accordingly, subsequent implantations were carried out in conjunction with a vigorous antiarrhythmic regimen. Serum potassium levels were determined preoperatively, and any tendency toward hypokalemia was corrected. Lidocaine (Xylocaine® HC1, Astra Pharmaceutical Products, Worcester, Massachusetts)[22,23] was used extensively. An initial loading dose of 80–100 mg was given early in the procedure. Thirty to forty mg were injected into the left atrium prior to intracardiac surgical manipulation. In addition, a continuous infusion of lidocaine was maintained throughout the procedure delivering 1 mg/minute. 100% oxygen was administered during all intracardiac procedures. Replacement transfusion with blood prewarmed to 37°C is accomplished via a catheter inserted in the left internal mammary vein. All animals used for implantation or as blood donors are typed and cross-matched. Thus far, all have been AB negative. These precautions combined with an improved technique of implantation to avoid acute blood loss resulted in the avoidance of ventricular fibrillation in the 15 procedures in which they were employed.

IMPLANTATION

Blood loss and coronary air embolism were frequent complications of left ventricular assist device implantation in our early studies. These problems were minimized by modifying the technique described by Bernhard. The modified procedure includes the use of a single balloon Foley catheter and the flooding of both the assist pump itself and the operative field with carbon dioxide.

A Dacron graft 20 mm in diameter is anastomosed end-to-side to the descending thoracic aorta at the level of the ninth rib and is occluded by a clamp applied close to the aorta to avoid intragraft thrombosis. The pericardium is opened widely to expose the entire left atrial appendage and the apex of the left ventricle. Following pericardiotomy, a Silastic ring is secured to the apical myocardium by an attached sewing cuff. One purse-string suture is placed at the base of the left atrium and another proximal to the Silastic ring (Figure 2). The outflow tube of the assist pump is connected to the aortic Dacron graft.

In initial procedures, a #24 double-balloon Foley catheter (Bardex®, C. R. Bard, Inc., Murray Hill, New Jersey) with an internal wire obturator was passed through an incision within the left atrial purse-string suture and the tip wedged into the ventricular apex. After inflation of the distal balloon a circular section of apical myocardium was excised with a circular coring knife until the intraventricular balloon was visualized. The balloon was immediately deflated allowing passage of the catheter through the apex of the left ventricle into the free pleural space. The purse-string suture and the proximal balloon were utilized to control bleeding. The wire obturator within the catheter was removed and the distal balloon of the catheter was inflated within the inflow tube of the left ventricular assist device to occlude the lumen. Carbon dioxide was then flushed through the catheter for two minutes to evacuate all air from the pump. Finally, the proximal balloon was deflated to allow manual insertion of the inflow tube into the left ventricle, with the catheter as a guide. A large silk ligature was used to secure the inflow tube in posi-

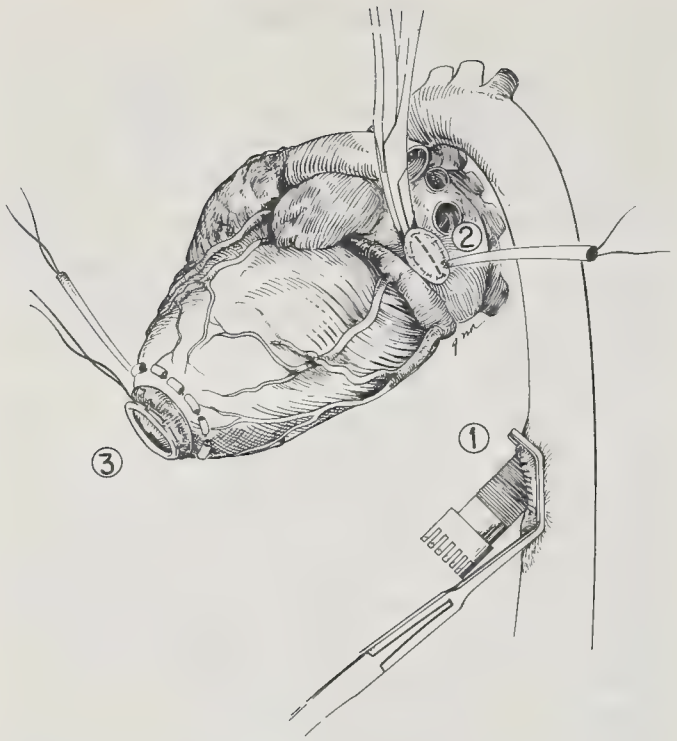


FIGURE 2.—Operative procedure for LVAD implantation in calves.

1. Outflow Dacron graft anastomosis to descending aorta.
2. Atrial purse-string suture.
3. Apical Silastic ring and controlling purse-string suture.

tion. The distal balloon was deflated and the catheter was withdrawn from the heart. Pumping was begun immediately. Despite the use of the purse-string suture and of the two balloons to control bleeding during the implantation, blood loss with this method was considerable.

In subsequent procedures the double-balloon catheter has been replaced by a #20 single-balloon Foley catheter (Bardex®, C. R. Bard, Inc., Murray Hill, New Jersey). The pump is flushed with carbon dioxide through the inflow tube. The entire operative field is flooded with CO_2 from a second line. The catheter is passed through the left atrium into the ventricle and the balloon inflated in the apex as in the earlier procedures. A circular section of apical myocardium is excised while blood loss is controlled by the intraventricular balloon (Figure 3). The inflow tube of the pump is inserted into the ventricle as the single balloon is deflated and withdrawn. When CO_2 ceases bubbling through

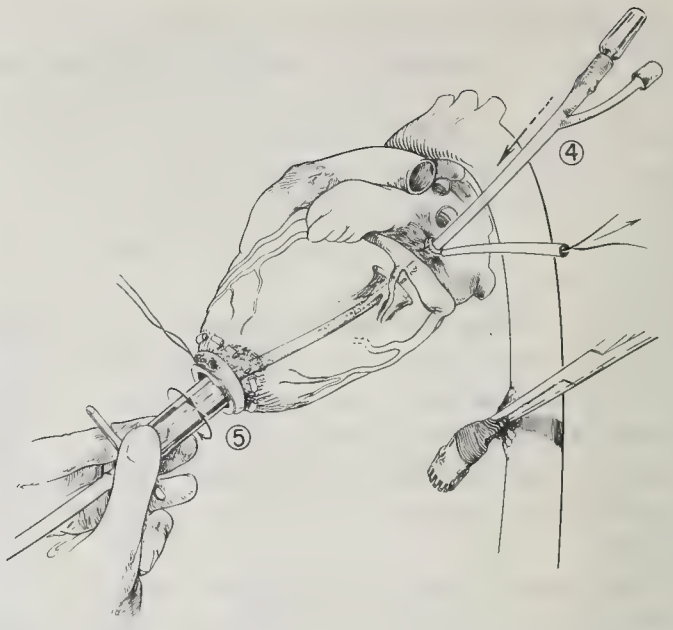


FIGURE 3.—Operative procedure for LVAD implantation in calves.

4. Single-balloon Foley catheter.
5. Excision of apical myocardium with circular coring knife.

the aortic Dacron graft and blood starts to appear the graft clamp is slowly released and pumping is initiated (Figure 4).

This modified technique has markedly decreased blood loss, since occlusion of the area of resected myocardium is virtually uninterrupted during insertion of the left ventricular assist device inflow tube, rather than being interrupted twice in the earlier technique. In addition, flushing of the pump with CO_2 via the inflow tube combined with CO_2 flooding of the operative field has proved effective in preventing coronary air embolism.

SUMMARY

The major operative hazards encountered early in our studies of left ventricular assist device implantation in the calf were (1) inadequate ventilation, (2) the marked irritability of the calf's heart and consequent occurrence of ventricular fibrillation during cardiac manipulation, (3) massive blood loss, and (4) coronary air embolism.

Technical procedures have been developed and applied with success in overcoming these problems. (1) Premedication with atropine sul-

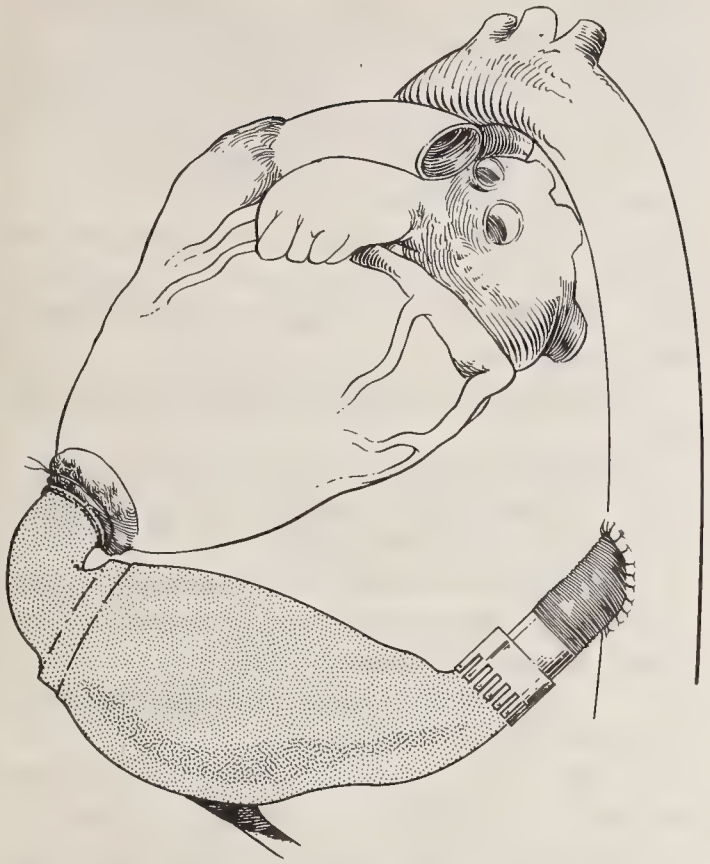


FIGURE 4.—LVAD in position.

phate, induction of anesthesia with small doses of sodium thiamylal, and maintenance in a light plane with Halothane inhalation have provided a satisfactory anesthetic program. Hypoxia, hypercarbia, and acidosis found at low ventilatory pressures of 15–20 cm H_2O were eliminated at higher pressures of 30–40 cm H_2O, without causing rupture of alveoli and pulmonary hemorrhage. Decompression of abdominal distention is often necessary and can be effectively carried out by percutaneous insertion of a large caliber needle into the rumen or by a temporary gastrostomy. Attempts to identify animals with preexisting pulmonary disease were disappointing. (2) A vigorous antiarrhythmic regimen involving preoperative correction of potassium deficiency and extensive use of lidocaine intraoperatively has thus far resulted in the avoidance of ventricular fibrillation. (3) Blood loss during implantation has been markedly diminished by the use of a single-balloon catheter technique and (4) coronary air embolism has been eliminated by flooding the pump itself and the operative field with carbon dioxide.

With increased use of the calf, present inaccuracies and gaps in the available information on this animal's physiology will be corrected. It is believed, that these modifications of previously employed procedures may offer an improved technical approach to successful left ventricular assist device implantation in the calf.

REFERENCES

1. FILLER, R. M., BERNHARD, W. F., BANKOLE, M., LAFARGE, C. G. and ROBINSON, T. C. An implantable left ventricular-aortic assist device. J. Thorac. Cardiovasc. Surg. 54:795, 1967.
2. BERNHARD, W. F., LAFARGE, C. G., HUSAIN, M., YAMAMURA, N. and ROBINSON, T. C. Physiologic observations during partial and total left heart bypass. J. Thorac. Cardiovasc. Surg. 60:807, 1970.
3. BONCHEK, L. I., TATOOLES, C. J. and BRAUNWALD, N. S. Experimental cardiac surgery in the calf: Techniques of anesthesia and operation. Ann. Thorac. Surg. 3:211, 1967.
4. Eds. ALTMAN, P. L. and DITTMER, D. S. Blood and Other Body Fluids, Washington, D. C., Federation of American Societies for Experimental Biology, 1961, pp. 27, 127.
5. DONAWICK, W. J., NARA, Y. and BAUE, A. E. Techniques for successful cardiopulmonary bypass in the calf. J. Surg. Res. 9:79, 1969.
6. BYRNE, J. J., BOYD, T. F. and DALY, A. K. Pasteurella infection from cat bites. Surg. Gyn. & Ob. 103:57, 1956.
7. DAVIS, B. D., DULBECCO, R., EISEN, H. N., GINSBERG, H. and WOOD, W. B. *Microbiology*, Harper & Row, New York, p. 802, 1967.
8. LYMAN, D. J., KWAN-GETT, C., ZWART, H. H. J., BLAND, A., EASTWOOD, N., KAWAI, J. and KOLFF, W. J. The development and implantation of a polyurethane hemispherical artificial heart. Trans. Am. Soc. Artif. Int. Organs 17:456, 1971.
9. GOODMAN, L. S. and GILMAN, A. *The Pharmacological Basis of Therapeutics*. Macmillan, New York, p. 525, 1965.
10. LARSON, R. E., MOFFITT, E. A. and MCGOON, D. C. Experimental cardiac surgery in calves, I. Anesthesia. J. Surg. Res. 3:101, 1963.
11. JENNINGS, S. The use of B. B. methyl ethyl glutarimide for terminating barbiturate anesthesia in calves. Vet. Rec. 70:494, 1958.
12. DONAWICK, W. J., HIREMATH, I. and BAUE, A. E. Anesthesia, ventilation and experimental thoracotomy in the calf. Am. J. Vet. Res. 30:533, 1969.
13. ROSS, J. N., JR., ALFREY, C. P., LYNCH, E. C. and KENNEDY, J. H. Hematologic observations during prolonged partial left ventricular bypass. Trans. Am. Soc. Artif. Int. Organs 17:195, 1971.

14. KLAIN, M., MRAVA, G. L., TAJIMA, K., SCHRIBER, K., WEBB, J., OGAWA, H., OPPLT, J. and NOSE, Y. Can we achieve over 100 hours' survival with a total mechanical heart? Trans. Am. Soc. Artif. Int. Organs 17:437, 1971.
15. TAKAGI, H., TAKANO, H. and AKUTSU, T. New series type left heart assist device with leaking outlet valve. Trans. Am. Soc. Artif. Int. Organs 16:431, 1970.
16. KWAN-GETT, C., BACKMAN, D. K., DONOVAN, F. M., JR., EASTWOOD, N., FOOTE, J. L., KAWAI, J., KESSLER, T. R., KRALIOS, A. C., PETERS, J. L., VAN KAMPEN, K. R., WONG, H. K., ZWART, H. H. J. and KOLFF, W. J. Artificial heart with hemispherical ventricles II and disseminated intravascular coagulation. Trans. Am. Soc. Artif. Int. Organs 17:474, 1971.
17. DONAWICK, W. J. and BAUE, A. E. Blood gases, acid-base balance and alveolar-arterial oxygen gradient in calves. Amer. J. Vet. Res. 29:563, 1967.
18. AKUTSU, T., MIRKOVITCH, V., TOPAZ, S. R. and KOLFF, W. J. Silastic sac type of artificial heart and its use in calves. Trans. Am. Soc. Artif. Int. Organs 9:281, 1963.
19. MCLAUGHLIN, R. E., TYLER, W. S. and CANADA, R. O. A study of the subgross pulmonary anatomy in various mammals. Am. J. Anatomy 108:149, 1961.
20. NELSON, R. J., MOHRI, H., DILLARD, D. H. and MERENDINO, K. A. Heterotransplantation of the human pulmonic valve to the calf pulmonic area. J. Thorac. Cardiovas. Surg. 52:863, 1966.
21. PETERS, RICHARD, M. The Mechanical Basis of Respiration, Little, Brown and Company, Boston, pp. 294 ff., 1969.
22. HARRISON, D. C., SPROUSE, J. H. and MORROW, A. G. The antiarrhythmic properties of lidocaine and procaine amide. Circulation 28:486, 1963.
23. ROSEN, M. R. and GRELBAND, H. Antiarrythmic drugs. Am. Heart J. 81:428, 1971.

DISCUSSION

CHAIRMAN MICHAEL P. KAYE: Thank you very much for your most interesting paper. This paper is now open for discussion.

KARL T. WEBER, JR., Birmingham: I'd like to ask you about the incidence of pulmonary hemorrhage with inspiratory pressures of 40 cm of water and why you feel that PO_2's of 228 are necessary as opposed to those of about 85 with 90 per cent saturation or better?

DR. MOLOKHIA: The PO_2 you see as 85 was associated with less than 100 per cent oxygen, which, of course, cannot be maintained for a long time. We didn't have the chance to study any pulmonary hemorrhage with high ventricular pressures because all these calves were long-term survivors and were not subjected to any biopsies following the operation.

HUGH CALDERWOOD, University of Florida: Have you tried positive and expiratory pressure in your ventilation experiments?

DR. MOLOKHIA: Yes.

DR. CALDERWOOD: And how did that turn out?

DR. MOLOKHIA: We couldn't sustain PO_2's for a long time.

DR. CALDERWOOD: What pressures were you using?

DR. MOLOKHIA: Up to 5 and 10 cm of water.

DR. CALDERWOOD: I suggest you might try a little bit higher in the calf.

DR. MOLOKHIA: Well, we are actually satisfied with the higher ventricular pressures. We haven't had any problem so far.

DR. CALDERWOOD: Have you determined a toxic dose for the lidocaine?

DR. MOLOKHIA: No, we have not, but we have had no trouble with lidocaine so far.

CHAIRMAN: Thank you very much. In a conversation I had last evening, there was some discussion of the use of insulin for its antiarrhythmic properties. Have you had any experience with this?

DR. MOLOKHIA: No, we haven't used insulin, but since we instituted our antiarrhythmic regimen, we haven't had any arrhythmias. Therefore, we haven't tried any other techniques.

JOHN C. NORMAN, Boston City Hospital: I want to thank Dr. Molokhia for presenting our paper. In response to Dr. Weber's question, I'd like to point out that most of the previous work indicates that if you use high inspiratory pressures in the young calf, you will get pulmonary hemorrhage. This varies with calf type. In the larger calves that we are using, with initial use of low inspiratory pressures and measurement of F_{IO2} and pulmonary functions, we end up with some hemorrhage. However, the calves of the size range we use are quite able to tolerate a high inspiratory pressure.

I might point out that our problem was a continued series of deaths from coronary embolism, hypotension, metabolic acidosis, respiratory acidosis, blood loss, and pulmonary failure. Our mandate at that time was to work out a

technique wherein clinical applications could be used in a nearly veterinary sort of approach. The ventricular fibrillation will obviously stop any experiment, and we have not been successful in using the standard DC countershocks, primarily because of the thick myocardial wall of the calf. However, we were able to skirt this problem by using an intensive antiarrhythmic regimen which involved continuous infusion of lidocaine and lidocaine bolus prior to emptying the apical left ventricular myocardial apex.

I think that the positive and expiratory pressures are pretty well established now on the clinical side. However, working out discriminate analyses of calves of varying states of maturity remains to be done.

Obviously we've not stressed the physiologic importance of being able to dissipate continually 50 watts of additional heat. This is work we are doing now and hope to present this spring. But if we have a 250 pound calf living over an eight or nine day period with serum hemoglobin levels less than 7 mg per cent, and we are able to dissipate about 85 per cent of 50 watts of heat directly into the circulating blood. This opens the way for a nearly direct prototype of a totally implantable nuclear fuel system. Obviously this has been our goal over the past 6–7 years in the program.

THE BABOON IN MEDICAL RESEARCH WITH PARTICULAR REFERENCE TO KIDNEY TRANSPLANTATION

G. P. Murphy*

Subhuman primates have specific applicability in current areas of research. The baboon has been found to have merit in the field of organ transplantation. Pertinent technical and related information concerning the use of this species in organ allotransplantation research is presented.

INTRODUCTION**

Research models in many fields have not necessarily reflected the degree of knowledge available or the rate of clinical activity. The development of the baboon as a suitable subhuman transplant model followed rather than preceded many human organ allotransplantation efforts.[1–7] In the case of human cardiac transplantation it played, in my opinion, a significant role.[8] Many questions in clinical transplantation remain to be answered. Some are never likely to be answered in the present patient settings because of the hazard to the patient. On the other hand, there remains much to commend the use, in selected circumstances, of subhuman primates such as baboons to deal with problems such as evaluation of antilymphocyte globulin effectiveness.[9] Obviously, there are other subhuman primates, such as the rhesus monkey, which have been most successfully employed in various ways by others.[10] However, there can be considerable technical problems with organ allografts using monkeys.[11,12] The present report will discuss pertinent aspects of the use of the baboon as an organ transplant model with particular reference to kidney transplantation.

* Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14203.

** These studies were supported in part by U.S.P.H.S. NIH Grant #FR 05648-05.

Vascular Aspects

One hundred and forty kidney transplants were done with the *en bloc* (aorta and inferior vena cava) and single artery method and were compared in relationship to immediate kidney function and the observed incidence of graft thrombosis occurring within the first 7 days after transplantation. Table I summarizes the apparent causes of death in these two series. The incidence of thrombosis was only slightly higher (34.1%) in the single kidney transplants as compared to the *en bloc* transplantations (28%) (Table I). This difference of only 6% is less than would be expected, considering the relative size of the vessels used for anastomoses in the two series. The *en bloc* grafts usually had an average vascular diameter of 3–4 times that of the single kidney transplants. In terms of the cross-sectional area at the anastomotic site, the difference between the two groups is even greater.

The clinical rejection rate of the two groups (50% for the single kidney transplants and 47.1% for the *en bloc* transplants) is virtually the same in the two series (Table I). In other animals, death resulted from the toxicity of some of the immunosuppressive agents used or from other systemic causes (Table I).

TABLE I.—*Causes of Death of 280 Baboon Kidney Transplants*

Type of Transplant	Total Done	Thromboses No.	Thromboses Percent	Rejection No.	Rejection Percent	Other Causes No.	Other Causes Percent
En bloc							
2 kidneys	140	40	28.0	66	47.1	34	24.9
Single kidney	140	48	34.1	70	50.0	22	15.9
Total	280	88		136		56	

TABLE II.—*Causes of Death in an Additional 350 Baboon Kidney Allografts*

Type of Transplant	Total Done	Thromboses		Rejection		Other Causes	
		No.	Percent	No.	Percent	No.	Percent
En Bloc							
One kidney	80	20	25	51	63.75	9	11.25
Single kidney with irrigation of recipient's vessels	120	30	25	75	62.50	15	12.50
Single kidney with aortic patch and irrigation of vessels	150	33	22	87	58.00	30	20.00
Total	350	83		213		54	

Table II compares the results of a further 350 transplants. These were done according to the variations on the two main groups as described. Where a single kidney was transplanted with the attached aorta and inferior vena cava segment, results were no worse than in the previously described groups where both kidneys were transplanted (Table II). The 3% lower thrombosis rate is not a significant improvement; however, it indicates that there is no definite advantage in transplanting both kidneys, as one kidney with normal function will sustain life in the baboon. In both instances, the related incidence of thrombosis observed with the variations of the single kidney transplant indicate an improvement over the previously obtained results. The incidence of thrombosis was 25% and 22% (Table II). The incidence of rejection relative to the decrease in the rate of thrombosis is higher in these three groups (Table II).

Graft thrombosis, when present in all the reported groups, was either arterial and venous or arterial only. No incidence of solitary venous thrombosis and graft clotting was observed. This reflects the lack of any technical difficulty with the venous anastomosis in either type of renal allograft technique.

The average ischemic time for the *en bloc* transplants was about 42 minutes. In the single kidney transplants, including those with an aortic patch, the average ischemic time was about 24 minutes. These operations were performed adequately by ten surgeons over a two year period. It is apparent, however, that vascular problems can still present serious difficulties in the use of baboons in some organ allografts. The problems are, however, less than those encountered with other primates.[11,12]

In experimental animals where the donor need not be sacrified, the direct arterial transplantation method and the time saved are obvious advantages of transplanting single kidneys.

Where only one kidney was transplanted with the *en bloc* method, the results achieved were very similar to those obtained when both kidneys were used (Tables I and II). Single kidneys with single vessels would seem, therefore, to be the most advantageous for experimental transplantation. Therefore, an attempt was made to lower the thrombosis by irrigating the segments of the recipients' vessels between the vascular clamps to wash out any blood which might give rise to eventual thrombosis. Possible clots were then flushed out by releasing the vascular clamps before the final suture was tightened and tied. This was thought necessary because the animals were not heparinized for the transplant procedure. These precautions slightly improved the results. The incidence of thrombosis in the group was 25% (Table II) as compared to 34.1% in the other single kidney transplants (Table I). This is an overall improvement of 9%.

A further modification lower the incidence of graft thrombosis to 22% in the single kidney transplants (Table I). This was achieved by utilizing an aortic patch to give a wider anastomotic opening. This method was of special value in some smaller animals with relatively smaller renal vessels. Combined with the described irrigating procedures, the achieved 22% thrombosis influence (Table II) was satisfactory when compared to the previous incidence of 34.1% (Table I). This was an overall improvement of 12%.

Cardiac Surgery and Cardiac Transplantation

The use of the baboon as a subhuman primate in experiments necessitating the use of a heart

lung machine has definite and very important advantages. Baboon blood groups correspond to the human ABO groups. The heart lung machine can thus be primed with compatible blood. Baboons also tolerate cardiopulmonary bypass well. Even lengthy perfusions of up to three hours using high flow rates of 100 to 125 ml/min/Kg body weight are followed by prompt and complete recovery. During experimental mitral valve replacements, no deaths occurred during operation in marked contrast to experience with mongrel dogs.[7] For cardiac transplantation, also, the baboon may be as good if not better than the mongrel dog.[7] Donors and recipients of suitable size can be selected for blood groups and leukocyte antigen compatibility. The whole procedure is easily accomplished through a left thoracotomy. The anatomy of the baboon heart and the great vessels is closely related to that of the human. The anastomoses of the atria, aorta and pulmonary arteries present no great problem provided adequate lengths of the donor preparations are obtained and fine suture material is used. The size of the heart corresponds closely to the body weight, simplifying the selection of donor-recipient pairs. Heparinization is achieved with doses similar to those used in humans and the heparin is effectively neutralized by Protamine sulphate. The bleeding tendency so commonly observed following cardiopulmonary bypass in dogs, has not been encountered in any baboon experiments.[7]

Bacteriological Aspects

The majority of this available material is based on a comprehensive study in 943 baboons previously reported elsewhere.[1,13]

Bacteriological specimens were taken from 943 baboons and include specimens of saliva, feces, urine, and skin scrapings. Mycobacterium tuberculosis was never found. Table III shows the incidence of some bacteria in the oral cavity of apparently healthy baboons. With the exception of five, all these animals were not in captivity long.

The incidence of Staphylococcus aureus and proteus species was found to be high. The incidence of staphylococci was astonishing, since it is far higher than that noted in humans

TABLE III.—*Incidence of Bacterial Species in the Oral Cavity of Baboons*

Bacterial Species	Percentage	Number
Staphylococcus aureus haemolyticus	61.8	576
Streptococci, beta-hemolytic	1.1	11
Enterococci	0.7	7
Escherichia coli	58.3	550
Klebsiella pneumoniae	32.2	304
Proteus	36.4	343
Paracolon	4.3	38
Pseudomonas aeruginosa	3.3	31
Alcaligenes faecalis	1.0	9

(± 20 percent) in South Africa. Antibiograms were performed on all those isolated. The highest rate of resistance occurred in the novobiocin and tetracycline groups. The antibacterial spectrum of novobiocin resembled that of penicillin G. Therefore, it was not surprising that most of the Gram-negative baboon flora were resistant to novobiocin. Tetracyclines are widely used in South African agriculture. This may be a selecting factor even in the free-living baboon flora.

Pathogenic micro-organisms of the fecal flora were found. Nineteen percent of all free-living baboons were carriers of enteropathogenic types of Escherichia coli. The most common types of enteropathogenic E. coli were 0 55, 0 119, and 0 26. The types 0 86, 0 111, and 0 127 occurred only sporadically.

Salmonella sundsvall was isolated from 52 baboons, not one of which showed signs of clinical disease. This type of salmonella seldom occurs in human beings in the Cape Province of South Africa, but in Canada and Mexico it has been described as the cause of gastroenteritis.[13] These were always sensitive to ampicillin. Ampicillin treatment administered preoperatively was, therefore, considered advisable to eliminate this latent but potent organism in baboons.

It is well known that monkeys and baboons carry shigellae, mostly without clinical signs. The incidence observed in our colony is similar to that reported in the series of 943 baboons, where Shigella flexneri type IV was isolated from 43; Shigella flexneri type II, from 25 and Shigella flexneri type V, from ten.[13]

Bacteriological findings in gallbladders are summarized in Table IV. It shows the difference in the incidence of Salmonella sundsvall in this flora and the fecal flora. Salmonella sundsvall

TABLE IV.—*Bacteriological Examination of 210 Gallbladders from Baboons (post-mortem)*

Organism	Number Infected
Salmonellae	
S. typhimurium	8
S. sundsvall	1
Diverse types producing gastroenteritis	7
Shigellae	
Sh. flexneri type II	1
Sh. flexneri type V	1
Enteropathogenic E. coli	
Type 026	3
Type 055	3
Type 0111	2
Type 0119	5
B proteus	
Pseudomonas	123
Klebsiella	
No growth	56

is possibly not able to cause bacteremia and, therefore, is found only rarely in the gallbladder.

About 33.3 percent of all animals were free from parasites; 46.4 percent carried Trichuris trichiura; 13 percent, trichostrongylus; 11.9 percent, Strongyloides stercoralis; 8.3 percent, ascaris; 10.3 percent, Entamoeba histolytica; 1.2 percent, dictocaulus; and 1.2 percent, paramaecium. Some animals had more than one parasite. Schistosoma was never found because all animals were trapped in that part of the country that is free from Bilharziasis. These organisms are of importance in that their presence must be taken into consideration in liver and other intestinal tract surgery.

Tissue Antigens

Information regarding baboons has been somewhat limited.[4,7] Balner and associates have extensive data on the monkey and chimpanzee.[10] Cohen has recently summarized the status of ABO erythrocyte typing in baboons.[14,20]

No naturally occurring isoagglutinins have been found in Papio cynocephalus and P. anubis.[15,16,17] However, isoimmunization has been used to produce typing reagents. Evidence is cited that A^P (P for Papio) and B^P groups determine four types of baboon blood.[18] The A^P–B^P blood group is distributed differently in the different species of baboons.[19] Since anti-A^P and anti-B^P sera contain other as yet undefined specificities, further study is necessary.

A. S. Wiener and J. Moor-Jankowski,[15,19] as well as others,[16,20] have reported that erythrocytes of baboons are not agglutinated by potent human A, B or H reagents. Grouping for human-like A–B–O antigen is done by inhibition tests of saliva for presence of blood group substances and by tests of sera for anti-A and anti-B agglutinins.

A group of 52 baboons (Papio papio, subgenus P. ursinus) were utilized in renal homotransplantation studies.[21] All animals were B^P-type in terms of simian (baboon) blood group substances. This may not be unexpected, since they represented a geographically isolated population with evidence of many years of inbreeding. Donor-recipient pairs of animals were matched on the basis of human-like A–B–O blood groups. Blood grouping studies were initially carried out by the laboratory of Wiener and Moor-Jankowski[17] and then by Brede and associates in South Africa.[21] No immunosuppressive drugs were used. Survival averaged 26.8 days for compatible renal allografts and 14.8 days for incompatible allografts with rejection and death of animals. Of special interest is a humoral antibody reacting with sheep and goat erythrocytes that appeared post-transplantation. High titers were noted in long survival "compatible" allograft recipients. Low titers were found in the "incompatible" transplanted recipients. However, we[21] considered the antibody similar to that described by Iwasaki, Talmadge and Starzl,[22] in post-renal allografted human recipients.

In recent studies Cohen[20] has evaluated HLA leukocyte distributions in relationship to available human antisera.

In Table V are shown the human-like leukocyte antigens encountered in highest frequency, as determined by tests with human reagent antisera.[20]

TABLE V.—*Human Leukocyte Antigens of Leukocytes of Baboon (Cape Chacma) Papio Ursinus—West Cape Region—South Africa*

Antigens Arranged in Order of Highest Frequency
HLA 1
4c (HLA 5)
(HLA 8) 7d, 4a (B11)
HLA 3
4b (B6)
HLA 4
HLA 2
4d (HLA 7)

TABLE VI.—*Human Leukocyte Antigens of Leukocytes of Baboon (Ape Chacma) Papio Ursinus—West Cape Region—South Africa*

Other Nomenclature	Antigen	Number of Animals with Antigen
B11	4a	10
B6	4b	7
HLA 5	4c	16
HLA 3	LA 3	9
HLA 4	LA 4	3
HLA 8	7d	10
HLA 1	LA 1	23
HLA 2	LA 2	2
HLA 7	4d	2

Microcytotoxicity test with Trypan Blue (method of Amos) employed. Reagent sera from NIH and RPMI.

The human-like antigens detected in highest frequency were HLA 1, HLA 5(4c), HLA 8(7d), and B 11(4a). The actual frequencies and the number of animals tested is presented in Table VI. The results are interesting to compare with those observed in chimpanzees and gorillas by Dorf and Metzgar.[23] They found HLA–1, HLA–3, HLA–7 and HLA–4 of highest frequencies in chimpanzees and HLA–2, HLA–1, HLA–3, HLA–4, and HLA–7 most frequent in gorillas. No reactions were obtained with HLA–5 and HLA–8 human reagents with chimpanzees or gorillas.

Balner, Van Leeuwen, Dersjant, Vreeswijk and Van Rood[24] found human-like leukocyte antigens 4a and 4b most frequent in chimpanzees.

There is a need for family studies and frequency analyses of baboon and human-like baboon leukocyte alleles comparable to those employed by Balner, Dersjant, Van Leeuwen, and Van Rood,[25] with rhesus monkeys. They found "rhesus" antigens 1a and 1b similar to human 4a and 4b, respectively, of the Van Rood system. However, "rhesus" antigens 1a and 1b, as well as 3–7 were not demonstrable on leukocytes of the baboon Papio cynocephalus.

Cross-absorption tests with human and baboon leukocytes and with human and baboon reagents are indicated and must be pursued in the future.

SUMMARY

This resume includes pertinent aspects of the use of the baboon as a research model, with particular reference to kidney transplantation.

Particular application depends on the specific point in question.[26] Serochemical baseline data is not adequate at the present time, but can be provided if adequate numbers of investigators seek use of these animals for related purposes. Cost, transportation and availability of numbers are moderate.[1,8,14] In a short period, a surprising amount of data in a specific field has been collected by a limited number of investigators.[1–8,13,14] But a few of these examples have been cited, and more undoubtedly will become available in other fields of research.[26]

ACKNOWLEDGEMENT

I wish to acknowledge my appreciation to colleagues who have provided great stimulus to research in this area, Dr. W. W. Scott, The Johns Hopkins Hospital, Professors A. J. Brink, J. J. W. Van Zyl, H. D. Brede, H. W. Weber, and J. N. DeKlerk of the University of Stellenbosch Faculty of Medicine, Cape Province, South Africa.

REFERENCES

1. VAN ZYL, J. J. W., DEKLERK, J. N., and MURPHY, G. P. Baboons in organ transplant research. Special Supplement to South African Medical Journal, 17: 1–105, Aug., 1968.
2. VAN ZYL, J. J. W., MURPHY, G. P., and DEKLERK, J. N. Baboons in transplantation research at the University of Stellenbosch. Lab. Primate Newsletter, 7:17, 1968.
3. GROENEWALD, J. H., MURPHY, G. P., WEBER, H. W., and BREDE, H. D. Biological methods of immunosuppression in the baboon. In *Infections and Immunosuppression in Subhuman Primates*. Eds. H. Balner and W. I. B. Beveridge, Scandinavian University Press, J. Jorgensen, Copenhagen, Denmark. pp. 211–216, 1970.
4. MURPHY, G. P., BOTHA, M. C., BREDE, H. D., WEBER, H. W., RETIEF, F. P., VAN ZYL, J. J. W., VAN ZYL, J. A., and DEKLERK, J. N. Correlation of tissue-typing tests in baboon renal allotransplants. J. Surg. Res. 9:19–28, 1969.
5. DEKLERK, J. N., MURPHY, G. P., VAN ZYL, J. J. W., VAN ZYL, J. A., WEBER, H. W., BREDE, H. D., and SCOTT, W. W. New methods of treatment for renal allotransplants using the baboon as a primate experimental model. J. Urol. 102:532–540, 1969.
6. WEBER, H. W., BREDE, H. D., RETIEF, F. P., RETIEF, C. P., VAN ZYL, J. A., GROENWALD, J. H., VAN ZYL, J. J. W., and MURPHY, G. P. Morphological and functional alterations noted after baboon renal allotransplantation. J. Urol. 101:465–476, 1969.
7. MURPHY, G. P., BREDE, H. D., COHEN, E., GRACE, J. T., JR. The Cape Western baboon in organ allo-

transplantation. Transplantation Proc., II, No. 4: 546–549, 1970

8. Brink, A. J., Ed. Organ Transplantation in South Africa (Symposium on Organ Transplantation 6–7, Oct., 1967), University of Stellenbosch Press, Stellenbosch, South Africa, 1967.
9. Murphy, G. P., Brede, H. D., Schoonees, R., Groenewald, J. H., Cohen, E., Amiraian, K., and Moore, G. E. Biological testing of antilymphocyte sera and globulin in the baboon. J. Surg. Oncology, 3:169–183, 1971.
10. Balner, H. In vivo evaluation of antihuman lymphocyte sera. Federation Proc. 29:117–119, 1970.
11. Herrmann, J. B., and Murphy, G. P. Renal transplantation in the rhesus monkey. I. Use of the American vascular stapler. Invest. Urol. 2:254–260, 1964.
12. Murphy, G. P., Melby, E. C., Mirand, E. A., Huser, H. J., Weldon, C. S. Renal transplantation in the rhesus monkey. VI. Observations in homotransplanted animals. Invest. Urol. 3:244–267, 1965.
13. Weber, H. W., Brede, H. D., Retieif, C. P., Retif, F. P., and Melby, E. C. The baboon in medical research: Base-line studies in fourteen hundred baboons and pathological observations. In *Defining the Laboratory Animal:* Proceedings of Symposium of the National Committee on Laboratory Animals, organized by the National Committee on Laboratory Animals and the Institute of Laboratory Animal Resources, National Research Council, Nat. Acad. Sci., Washington, D.C. pp. 528–549, 1971.
14. Cohen, E. The erythrocyte blood group systems—grouping, matching, and role in transplantation. In *The Baboon in Organ Transplantation.* Ed. G. P. Murphy, Karger Press, Basel, Switzerland (In press), 1972.
15. Moor-Jankowski, J., Wiener, A. S., and Gorden, E. B. Blood groups of apes and monkeys. I. The A–B–O blood groups in baboons. Transfusion, 4: 92–100, 1964.
16. Podliachouk, L., and Dubouch, P. The A–B–O groups in a baboon colony in France. Vox Sang. 18:467–470, 1970.
17. Wiener, A. S., and Moor-Jankowski, J. Blood groups in anthropoid apes and baboons. Science, 142:67–69, 1963.
18. Moor-Jankowski, J., Wiener, A. S., Kratochvil, C. H., and Fineg, J. Seroprimatology of chimpanzee blood group distribution as racial characteristic. Science, 152:219–220, 1966.
19. Moor-Jankowski, J., Wiener, A. S., and McRitchie, R. Isoantibodies produced in chimpanzees by cross-immunization with gibbon red cells. Int. Arch. Allergy, 34:386, 1968.
20. Cohen, E., Gregory, S. G., Dozier, A., Groenewald, J., and Murphy, G. P. Human-type erythrocyte A–B–O group and leukocyte antigens of *Papio ursinus,* South Africa. Medical Primatology, 1970, Proc. 2nd Conf. Exp. Med. and Surg. in Primates. New York, 1969, pp. 148–152 (Karger, Basel 1971).
21. Murphy, G. P., Weber, H. W., Brede, H. D., Retief, F. P., Retief, C. P., Van Zyl, J. A., Groenewald, J. H., and Van Zyl, J. J. W. The significance of human ABO blood groups in the survival of untreated baboon renal allotransplants. Amer. Surg. 35:292–300, 1969.
22. Iwasaki, Y. Talmadge, D., and Starzl, T. E. Humoral anti-bodies in patients after renal homotransplantation. Transplantation, 5:191–206, 1967.
23. Dorf, M. E. and Metzgar, R. S. The distribution of human HLA antigens in chimpanzees and gorillas. In Goldsmith and Moor-Jankowski, *Medical Primatology,* 1969, (Karger, Basel, 1971).
24. Balner, H., Van Leeuwen, A., Dersjant, H., Vreeswijk, W., and Van Rood, J. J. Leukocyte antigens of chimpanzees and rhesus monkeys. In Goldsmith and Moor-Jankowski, *Medical Primatology,* 1970, pp. 31–43 (Karger, Basel 1971).
25. Balner, H., Dersjant, H., Van Leeuwen, A., and Van Rood, J. J. Histocompatibility testing in primates. Vox Sang. 11:306–314, 1966.
26. Vagtborg, H., Ed. *The baboon, and annotated bibliography.* The Southwest Foundation for Research and Education, San Antonio, Texas, pp. 1–642, 1964.

DISCUSSION

Chairman Frank J. Veith: Dr. Murphy's excellent presentation is now open for questions or discussion. I have a question myself. Apparently positive results in testing antilymphocyte globulin (ALG) or antilymphocyte serum preparation in monkeys indicates that the serum will be effective in man. However, Dr. Nagerian's serum, which has been shown to be effective in human volunteers with multiple scherosis, did not have an effect on Dr. Balner's monkeys. Therefore, a negative response in monkeys would not necessarily mean the serum was no good in man. What would be your comment to that?

Dr. Murphy: It is always difficult and perhaps dangerous to talk about somebody else's research. I can only say that we have not found this to be so. Dr. Balner has been very generous with his time and resources in testing ALG and, on occasion, has been requested to give information based on one or two animals or one dose. My point today was that you cannot take the same dose in man and animals and effectively compare it. If there's a negative result, we take a little more time and effort, varying the doses by numerous degrees (up to ten times) to make sure that it really is negative.

PRECONDITIONING, IMPLANTATION AND POSTOPERATIVE CARE OF THE ARTIFICIAL HEART RESEARCH CALF

J. L. Peters, F. M. Donovan, J. Kawai,
C. S. Kwan-Gett, H. H. J. Zwart and W. J. Kolff*

In the last two years, seventy total heart implantation experiments have been performed in the calf. Emphasis has been to develop a preconditioned calf program to provide a standardization calf with a known growth and clinical history prior to implantation. Durability and short-term mock circulation testing procedures are performed on the artificial heart prior to implantation to assess its durability and functional characteristics. Implantation procedures have been developed to minimize trauma and blood damage and thereby more accurately separate the effects of implantation from the pumping of the artificial heart. These include deep hypothermia with circulatory and respiratory arrest during implantation of the artificial heart, and double bypass in which the natural ventricles are excised after the artificial heart is anastomosed and is maintaining the circulation. Two types of experimental procedures are used to study the artificial heart *in vivo*. One is the hemodynamic evaluation of the artificial heart in which multiple pressure recordings are made. Another procedure consists of one or two pressure recordings to reduce disturbances to the animal and allow long survival. Postoperative care of the calf with an artificial heart includes continuous monitoring of the clinical condition and physiological studies of the cardiovascular, pulmonary and renal systems. Autopsy examinations are performed on the suspended animal by removing the lateral rib cages in order to assess the fit of the artificial heart, possible vessel kinking and future design criteria. To date a calf has survived 260 hours with a total artificial heart. This calf supported itself, ate and drank, showed interest in its surroundings and required no respiratory support for eight days. The use of systematic and standardization procedures for preconditioning of calves, implantation of the artificial heart and postoperative care have produced long survivors and new design criteria for future artificial hearts.

INTRODUCTION**

In 70 total heart implantations in calves in the last two years in the laboratory of W. J. Kolff, methodologies have been developed to improve the use of the calf as a model for artificial heart research. The emphasis has been 1) to improve the quality of the calf prior to delivery to our facility; 2) to develop better methods of surgical implantation in order to minimize trauma and thereby more easily separate the effects of pumping of the artificial heart from those of implantation; and 3) to evaluate and develop techniques of postoperative care and data acquisition.

CALF PRECONDITIONING

In order to evaluate the effects of pumping by a total artificial heart in the calf, it is necessary to have a calf with a known clinical and developmental history. Since an artificial heart experiment is expensive, a calf with pneumonia, lung worms, dehydration and other pathological conditions (as found in stockyard calves) would increase the probability of uncontrolled variables and failure of the experiment (particularly a long survival experiment) from causes unrelated to the pumping of the artificial heart. In 1970, in conjunction with a local research animal supplier (Mr. Tom Imlay, Animals for Research, 4996 So. Redwood Rd., Murray, Utah 84107), we established a preconditioned calf program. The environment for these calves was quite extensive. Four to five hundred calves (98% Holstein and 2% other breeds) were raised simultaneously in specially designed growing nurseries and pens. The calves were obtained at birth from dairies within the Utah area. The nursery where the newborn calves were housed could hold 300 calves at one time. These calves were in individual elevated pens with controlled temperatures and humidity and fed on a combination of milk and supplemental grain—alfalfa pellets fortified with vitamins. Music was played at intervals to keep the young

* Department of Surgery, Division of Artificial Organs, University of Utah, College of Medicine, Salt Lake City, Utah.

** Supported by: N.H.L.I. (Medical Devices Applications Program), NIH Contract No. NIH 69-2181; and Program Project Grant from NIH, N.I.L.I., H.E. 13738-01A1 to the University of Utah, Dr. W. J. Kolff, Principal Investigator.

calves calm. These calves were immunized against calf diseases (Salmonella, Enterotoxemia, Clostridium Perfringens (types C & D), Parainfluenza (type 3), Infectious Bovine Rhinotracheitis, Clostridium Chauvoei), and had a medical record chart and a food intake chart. When the calves were weaned at approximately two months of age, they were transferred to growing pens and placed on a free choice diet of grain, alfalfa pellets and water (constantly running). The growing pens were half straw bed and half cement with flushing troughs for daily removal of waste. The calves in the growing pen gained 1 to 2 pounds in total body weight per day and were kept in quarantine as no other animal traffic was allowed at the facility. They were regularly evaluated by a veterinarian.

For artificial heart research calves, it is necessary to have base line blood chemistry which was performed on our calves usually twice prior to implantation. The routine control blood chemistry data included calcium, sodium, potassium, inorganic phosphate, blood sugar, blood urea nitrogen, uric acid, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase, serum glutamic oxaloacetic transaminase and fibrinogen. Hematology evaluation consisted of a complete blood count, platelet count and hematocrit. Other determinations performed on occasion were plasma hemoglobin, norepinephrine, epinephrine and 17–OH steroids (compounds F & B) and when the calves were delivered to our division, lung function studies were performed (tidal volume, O_2 consumption, respiratory rate, minute ventilation and functional residual capacity).

The results of this project provided a research calf that was raised under controlled conditions, diet, immunizations, temperature and humidity, and was delivered to our facility with a medical record card and base line blood chemistry data. The use of these calves provided disease-free calves of the same breed, approximately the same age and weight, for implantation of the artificial heart and evaluation of its effects.

EXPERIMENTAL PROCEDURES

One week prior to an artificial heart experiment, the principal investigators have experimental planning sessions to define: 1) the purpose or purposes of the experiment;)2 what experimental procedures will be performed; and 3) individual responsibilities. A preliminary protocol is written and distributed to all members of the research team for their suggestions and criticisms. (This sometimes requires another discussion session before the final protocol is written.) Usually, the day before the experiment, all members of the research team meet and go over the experimental protocol. At this time, the postoperative intensive care calf sitters are scheduled for a period of one week in advance.

Heart Testing

The Kwan-Gett hemispherical artificial heart[1] was used in most of our total heart replacement experiments during the past year. Each ventricle has a rigid circular base attached to a hemispherical diaphragm made of 0.030 inch dacon-reinforced silicone rubber. A 0.060 inch dacron-reinforced silicone rubber housing with inflow and outflow connectors fits over the diaphragm-housing junction. Outflow and inflow valves used include silicone rubber flap valves, tricuspid outflow valves and Wada-Cutter valves.

Figure 1 illustrates one cardiac cycle of one ventricle in which filling pressure regulates inflow rate to the heart and displacement of the diaphragm during diastole (70% of the cycle). The heart responds to Starling's Law with an increase in filling pressure causing an increase in cardiac output. During systole, air pressure from an external source displaces the diaphragm and blood is ejected through the aortic valve (30% of one cycle). Both right and left ventricles are operated simultaneously at a fixed rate of 120 b/min and at 4 psi and 6 psi driving pressure respectively. Surfaces used as the intima of the artificial heart have included smooth silicone rubber and silicone rubber lined with dacon fibrils.

Before an artificial heart is implanted, it is subjected to two types of tests *in vitro*. One

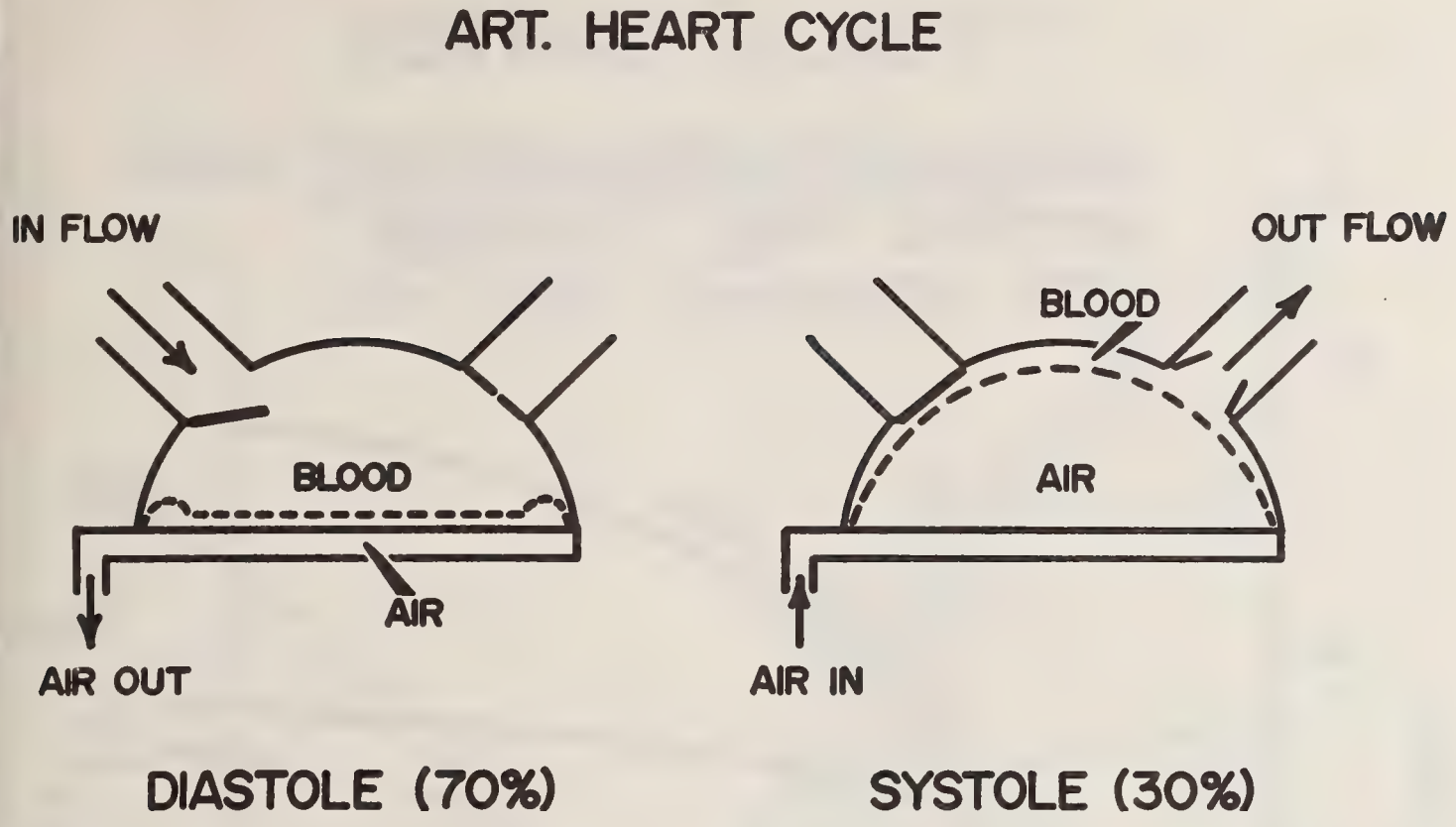


FIGURE 1.—Schematic illustrating cardiac cycle of one Kwan-Gett, air driven diaphragm artificial heart. Maximum stroke volume is 100 cc.

test is a simple determination of the durability of the heart. The artificial heart is placed on a mock circulation connected to a drive system and allowed to pump continuously against an arterial pressure of 100 mm of Hg with an atrial pressure adjusted to produce a cardiac output of approximately five liters/min. We have had one Kwan-Gett ventricle on a test of this type for fourteen months without failure. The second test is a determination of the hemodynamic function of the artificial heart. This test is also conducted on a mock circulation. The effects of atrial pressure, arterial pressure, heart rate and duration of systole on the cardiac output are determined. A sample of the function curves produced from these tests on a Kwan-Gett heart are shown in Figure 2. The effect of allowing the heart to exhaust air into a vacuum of 10 mm Hg as compared to exhausting to atmosphere is clearly shown. A set of function curves for a Donovan spherical heart exhausting to atmosphere is shown in Figure 3. This allows us to completely understand the hemodynamic response of the heart before it goes into the animal, and illustrates the differences in function curves with different designs of artificial hearts (i.e., maximum cardiac output increased with larger spherical artificial heart). This knowledge allows us to eliminate most of the pressure measurements which would be necessary to control the heart without pre-implantation function testing. For example, we can easily maintain an animal with the artificial heart implanted using only one pressure line for an arterial pressure and one pressure line for the central venous pressure.

Artificial Heart Implantation Procedures

In order to minimize surgical trauma and blood damage, two implantation methods, other than cardiopulmonary bypass, have been developed. The rationale for not using cardiopulmonary bypass in all experiments was to evaluate the immediate effects of pumping of the artificial heart independent of hemolysis and decreased coagulation factors which result from use of the heart-lung machine.

On some occasions, when cardiopulmonary

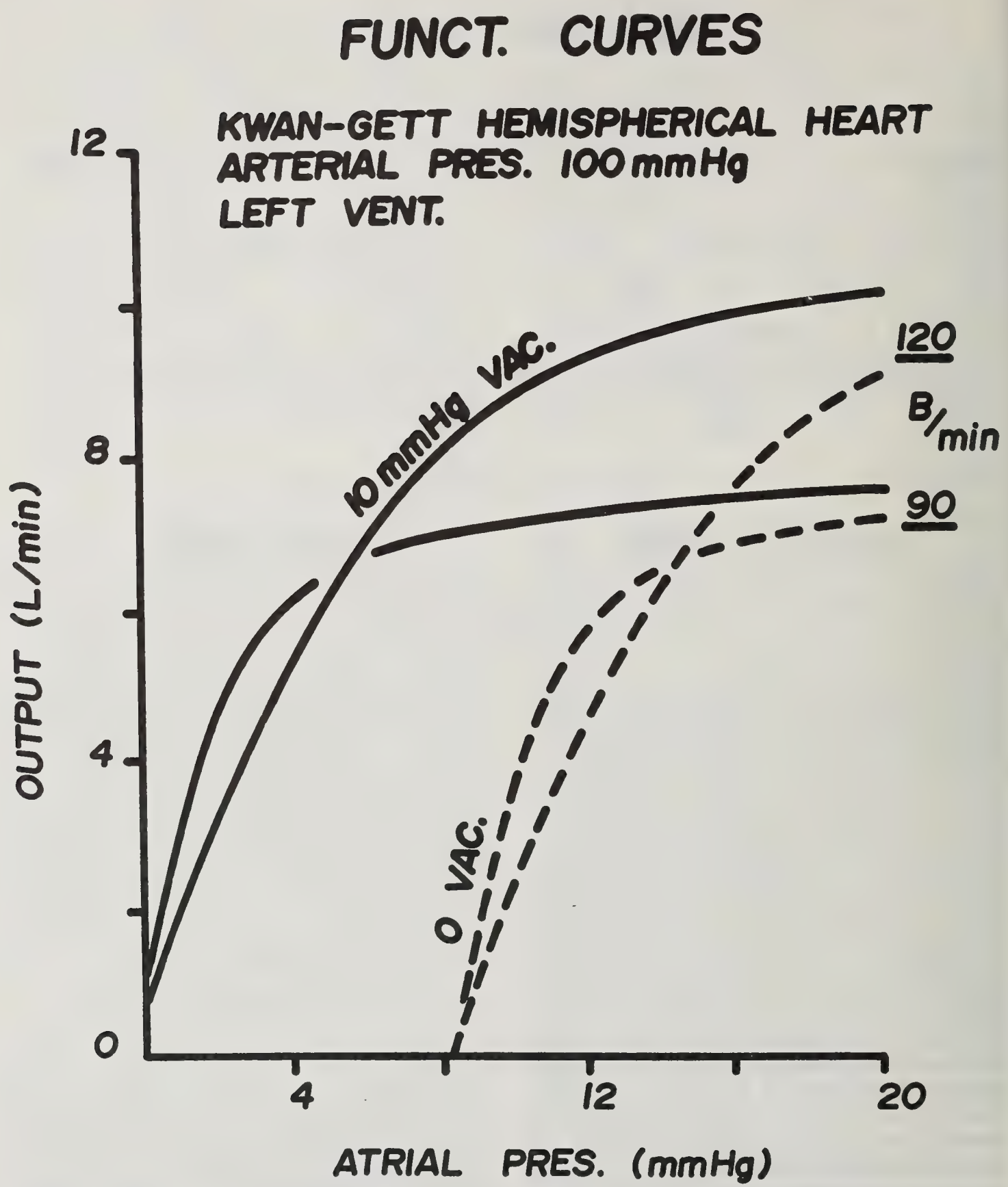


FIGURE 2.—Function curves of a Kwan-Gett hemispherical heart with and without vacuum applied during diastole. Curves for two heart rates are illustrated.

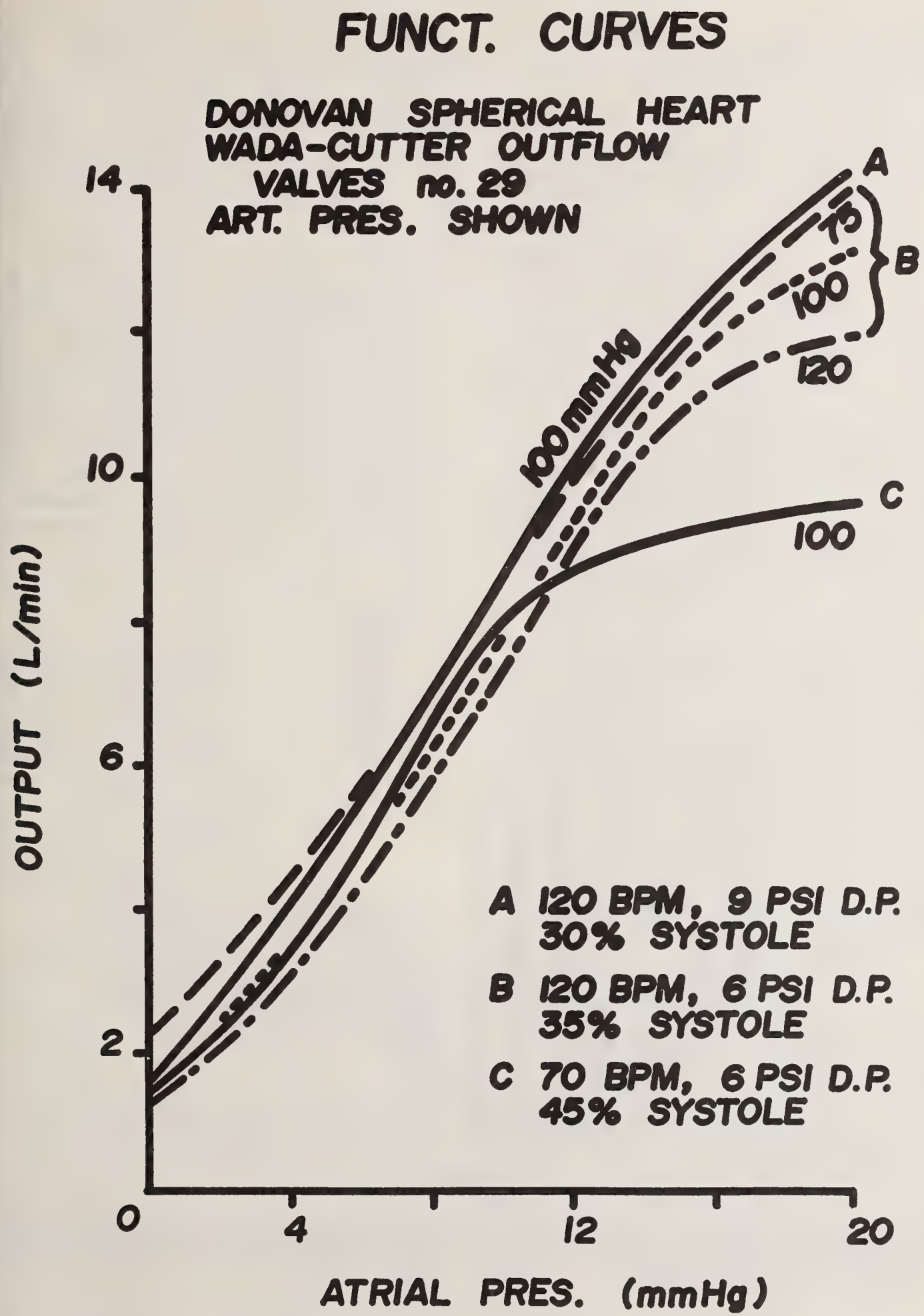


FIGURE 3.—Function curves of a Donovan Spherical Heart under different driving conditions. (Rate, driving pressure, % systole, outflow pressure). Maximum stroke volume is 150 cc.

bypass is employed with the calf, perfusion and blood pressure are inadequate and pressor agents have to be employed.

Deep Hypothermia

Dr. Jun Kawai introduced deep hypothermia in our laboratory as a method of implanting the artificial heart.[2,3]

The fasted, pre-conditioned calf is induced with scopolamine (0.015 mg/kg) and brevane (5 mg/kg), intubated and placed on a Bird Respirator (Mark 14), with halothane gaseous anesthesia. The calf is then instrumented with ECG electrodes, femoral arterial blood pressure catheter, external jugular vein catheter (for Ringer's lactate solution infusion) and a rectal temperature probe. The calf is placed in an ice bath and cooled to a rectal temperature of 24° C (range 22.5°—25° C). Figure 4 illustrates the changes in heart rate and blood pressure during cooling. On the operating table, cooling is maintained by ice bags on the head, neck and groin. The chest is opened by a midsternal split and the great vessels dissected and ligated after heparin (1 mg/kg) is given. Once the rectal temperature has decreased to 21° C, blood is expelled from the natural heart and with hyperventilation, from the pulmonary circulation. Both circulatory and respiratory arrest are maintained during the implantation of the artificial heart which usually takes 45 minutes. The artificial heart is pumped slowly at first and at low pressures in order to detect and repair blood leaks and to prevent the pulmonary and systemic circulation from being exposed to sudden high pressures and flows. Protamine sulfate is administered (1.5 mg/kg), four chest drainage tubes are inserted and the chest is closed. The calf is then placed in a warm water bath (40° C) until the rectal temperature reaches approximately 34° C (see Figure 4 for blood pressure changes during rewarming), after which the calf is placed in a specially

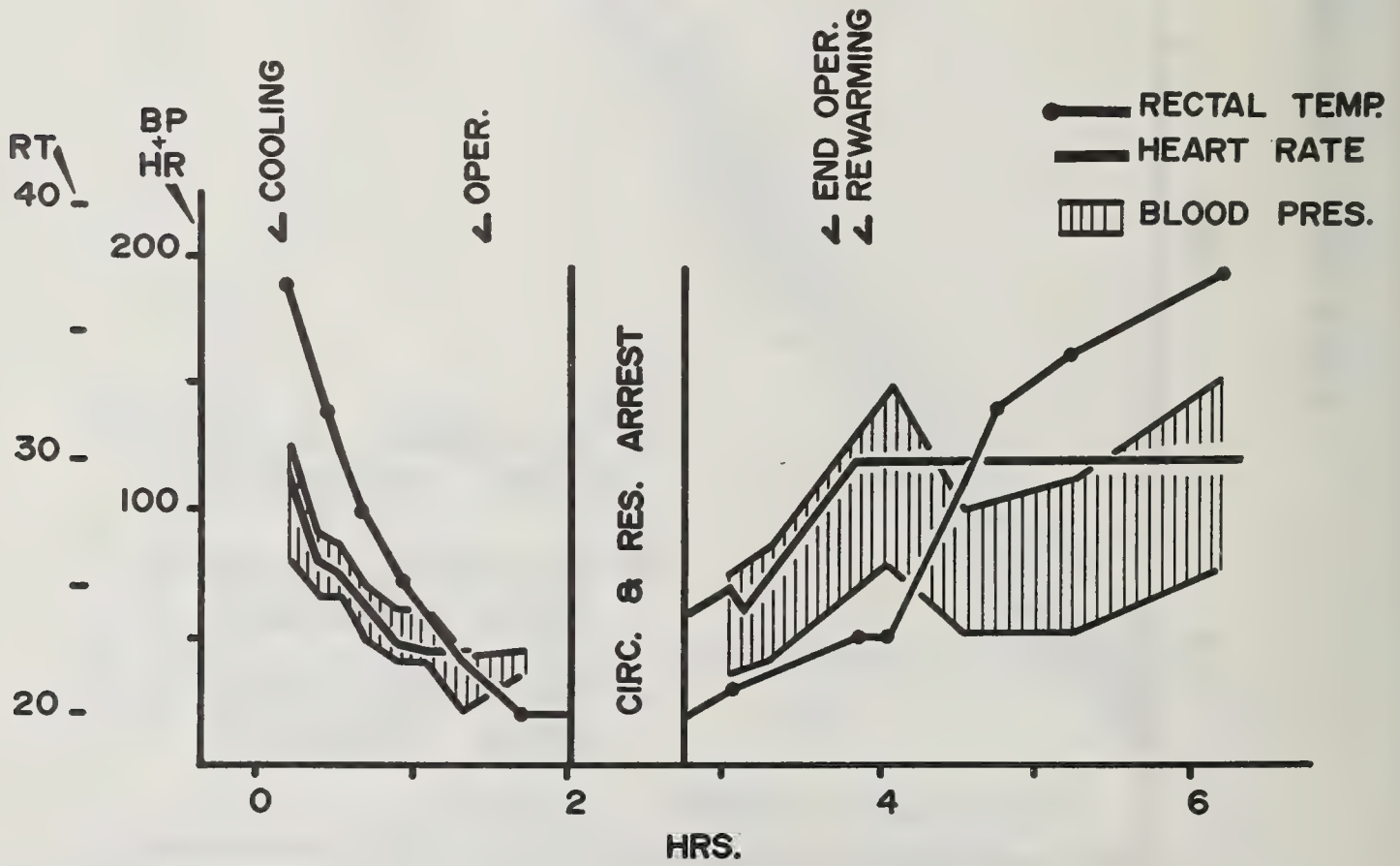


FIGURE 4.—Utilization of deep hypothermia to implant the artificial heart. Calf is cooled to approximately 20° rectal temperature at which temperature the natural heart is excised and replaced with an artificial heart during circulatory and respiratory arrest.

designed cart. The critical variables to monitor after implantation of the artificial heart under deep hypothermia are 1) arterial pH which tends to acidosis unless corrected during rewarming; 2) arterial pO_2 and arterial oxygen saturation which will decrease if the pH and respiratory support during rewarming are not carefully controlled; and 3) arterial pCO_2.

Double Bypass

A second technique of implantation of the artificial heart without cardiopulmonary bypass, was developed by Dr. Clifford Kwan-Gett.[4] In this method the calf, after anesthesia, is positioned midway between the dorsal and right lateral position for a fourth intercostal thoracotomy. The aortic graft is anastomosed to the descending aorta and the pulmonary graft is anastomosed to the pulmonary artery. The calf is then positioned on the dosal surface and a transverse thoracotomy completed. A left atrial connector is anastomosed and the left ventricle is attached and primed for pumping. In a like manner, the right ventricle is attached to the right atrial connector. After ventricular fibrillation is induced and both artificial ventricles are maintaining the circulation, the pulmonary artery and aortic roots are clamped, divided and sutured. The ventricles are then clamped and removed. The two artificial ventricles are then fitted into the middle of the thorax and attached to each other. Chest tubes are inserted and the chest is closed.

Cardiopulmonary Bypass

The standard method for open-heart surgery, cardiopulmonary bypass is used routinely in our laboratory along with our other procedures.[4] It is particularly useful for initial evaluation of a new artificial heart for which fit in the thorax and vessel connections are not standardized. It allows for direct end-to-end anastomosis of the great vessels as compared to the double bypass technique which utilizes end-to-side anastomosis. It allows more time for implantation compared to the deep hypothermia method (limit of circulatory and respiratory arrest of one hour) which utilizes direct end-to-end anastomosis quick connects.

A Bentley oxygenator is used with a prime of 2000 ml Ringer's lactate solution and 1000 ml of Dextran 40. Blood drainage is from the inferior vena cava by way of the right atrium and the superior vena cava by way of the right jugular vein. Blood is returned to the right femoral artery. The duration of total bypass varies from one to three hours with an average of approximately one and one-half hour.

PHYSIOLOGICAL MONITORING

Cardiovascular

There are two different types of experiments performed in this laboratory. In one type, we monitor central venous pressure, right atrial pressure, right ventricular pressure, pulmonary arterial pressure, left atrial pressure, left ventricular pressure, aortic pressure and femoral arterial pressure. In this type of experiment, the cardial output of the right heart is sometimes also monitored. Figure 5 shows the hemodynamic measurements made during one of these experiments. The purpose of this experiment, which is usually performed using a new design of artificial heart, is to determine as accurately as possible the hemodynamic response of the artificial heart *in vivo*. Once this has been accomplished for a particular design, we usually use the second type of experiment in which the only pressure measurements made are central venous pressure measured by a catheter inserted through the femoral vein and femoral arterial pressure. We do not use flow meters in these experiments. The purpose is to reduce the disturbance to the animal to a minimum. We have found that this allows us to obtain longer survival. The arterial pressure for a long survivor is shown in Figure 6 and composite plots of central venous pressure are illustrated in Figure 7.

The ECG is monitored with safety-pin electrodes externally prior to cutting out the natural heart. This is especially helpful during deep hypothermia to assess heart rate. Figure 8 illustrates a composite of atrial electrical activity in four calves measured with platinum wire electrodes sutured to the reminant natural atria. The electrical activity of the reminant atria attached to the artificial heart continues despite the removal of the coronary blood supply.

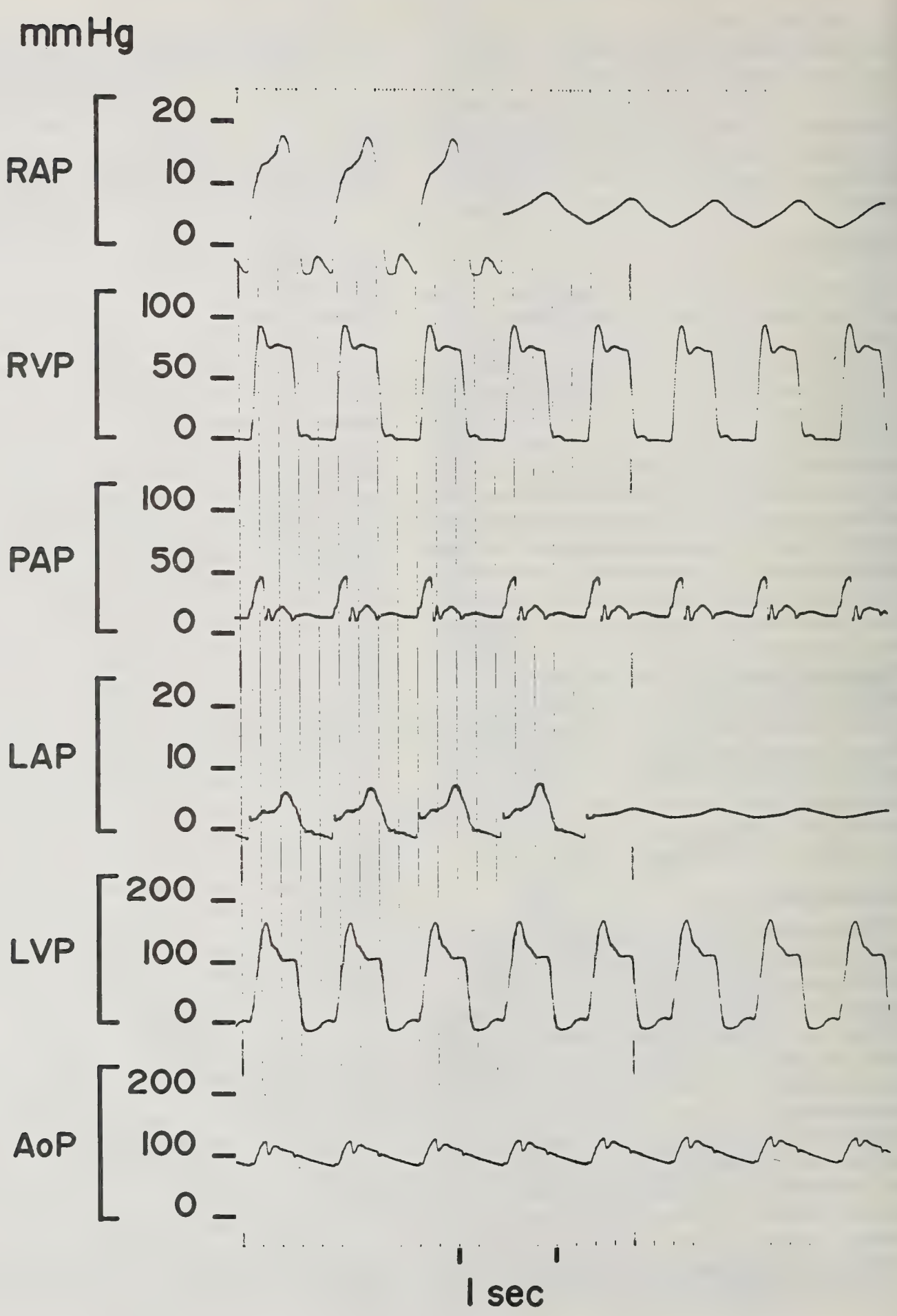


FIGURE 5.—Blood pressure in a calf with an artificial heart—hemodynamic evaluation.

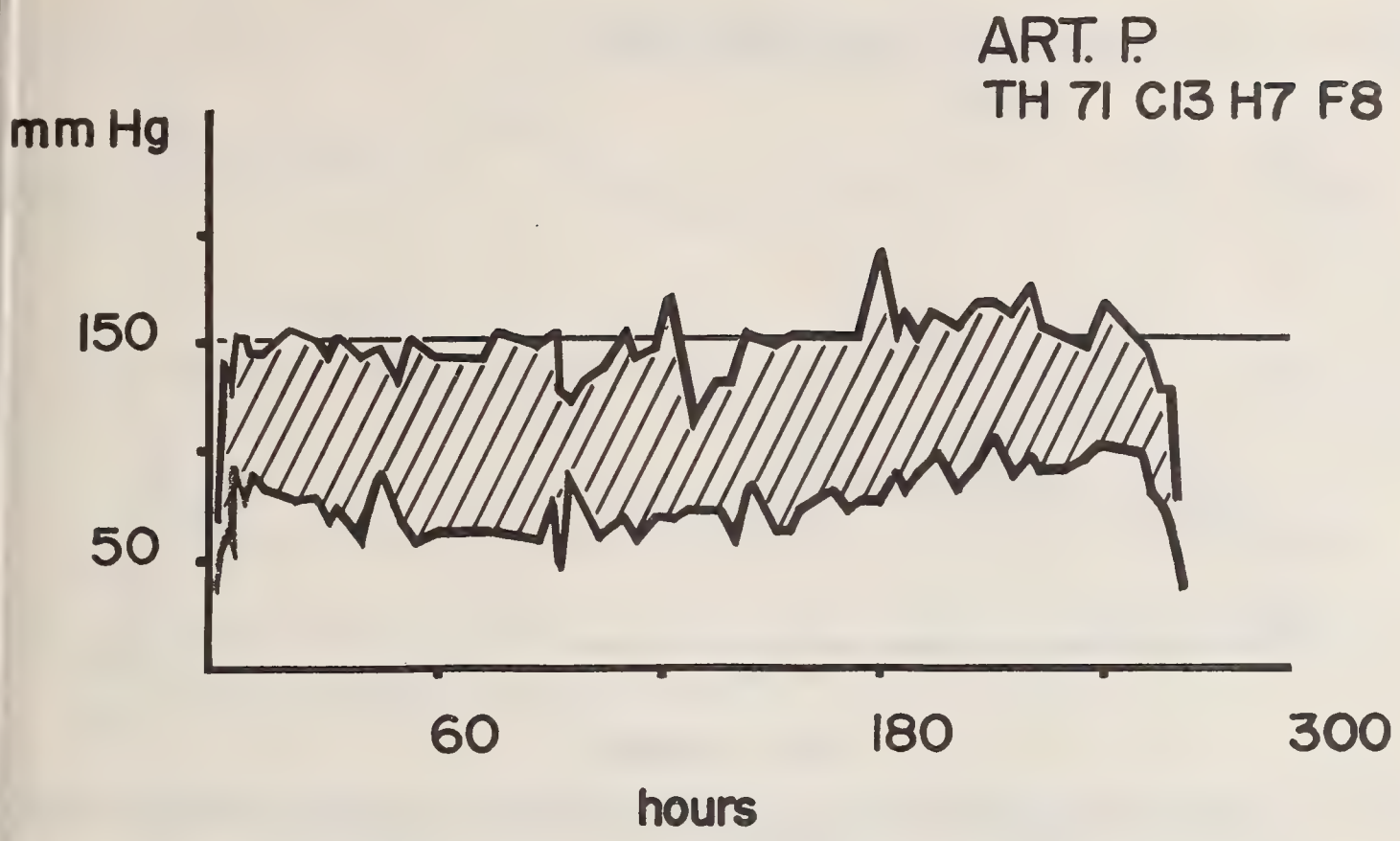


FIGURE 6.—Arterial pressure in a long surviving calf with an artificial heart.

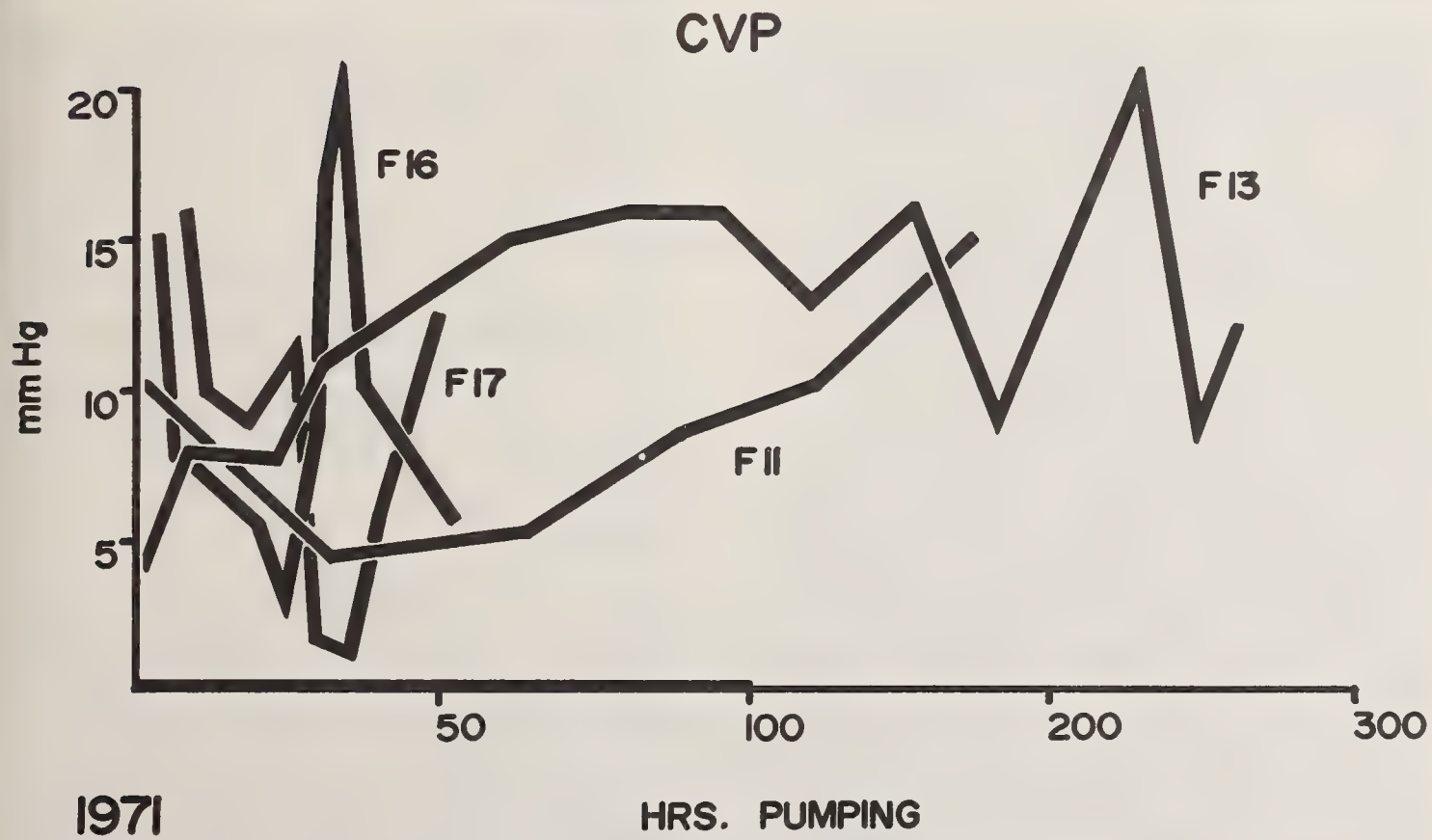


FIGURE 7.—Composite of central venous pressure in four calves with time of pumping of the artificial heart.

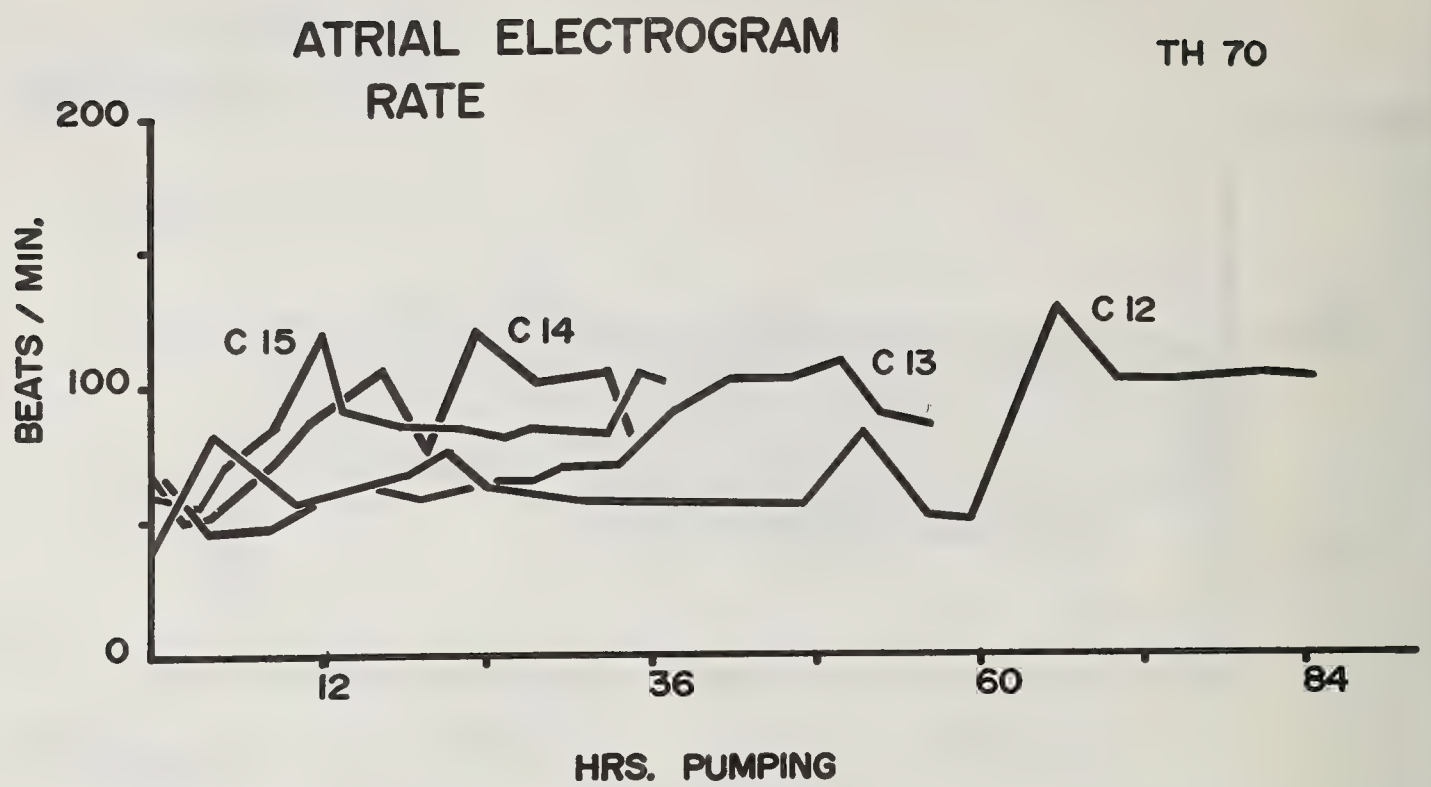


FIGURE 8.—Composite of the atrial electrogram in four calves measured from the remnant natural atria attached to the artificial heart. This signal can be used to pace the artificial heart.

Blood Chemistry

The following blood chemistry determinations are normally obtained in our long-surviving animals. Potassium, sodium, calcium, phosphate, blood sugar, blood urea nitrogen, uric acid, cholesterol, total protein, albumin, globulin, albumin to globulin ratio, total bilirubin, alkaline phosphatase, LDH, SGOT, and free plasma hemoglobin. A plot of plasma—Na, Ca and K is shown in Figure 9. In addition to the blood chemistry measurements, we also periodically obtain platelet count, hematocrit and clotting time. A composite plot of the platelet count is shown in Figure 10 and a plot of blood volume and hematocrit is shown in Figure 11. In twelve of our total heart experiments, studies have been made of prothrombin time, partial thromboplastin time, and thromboplastin time. Blood clotting factors I, V, and VIII have been determined in these twelve experiments as well as platelet adhesiveness, platelet survival time, fibrin degradation products and clot lysis products.[5,6,7] Other blood chemistries obtained include plasma catecholamines and steroids, some results of which are shown in Figures 12 and 13.

Pulmonary

Blood gases are measured approximately every two hours. The determinations are arterial pO_2, oxygen saturation, blood pH and arterial pCO_2. Figure 14 shows arterial pO_2 and pCO_2 for a long survivor. After surgery, approximately each 24 hours, determinations are made of the respiration rate, tidal volume, minute ventilation, oxygen consumption and functional residual capacity. The cardiac output is estimated using the Fick method. Tidal volume, oxygen consumption and functional residual capacity are shown in Figure 15 for a long-surviving calf.[8]

Renal Function

In order to determine the condition of the kidneys, the following measurements are made: serum creatinine, blood urea nitrogen, urine creatinine, creatinine clearance, urea clearance and urine specific gravity. Microscopic examination of the urine is conducted to determine the content of red blood cells, white blood cells and casts. Urinary potassium is also determined.

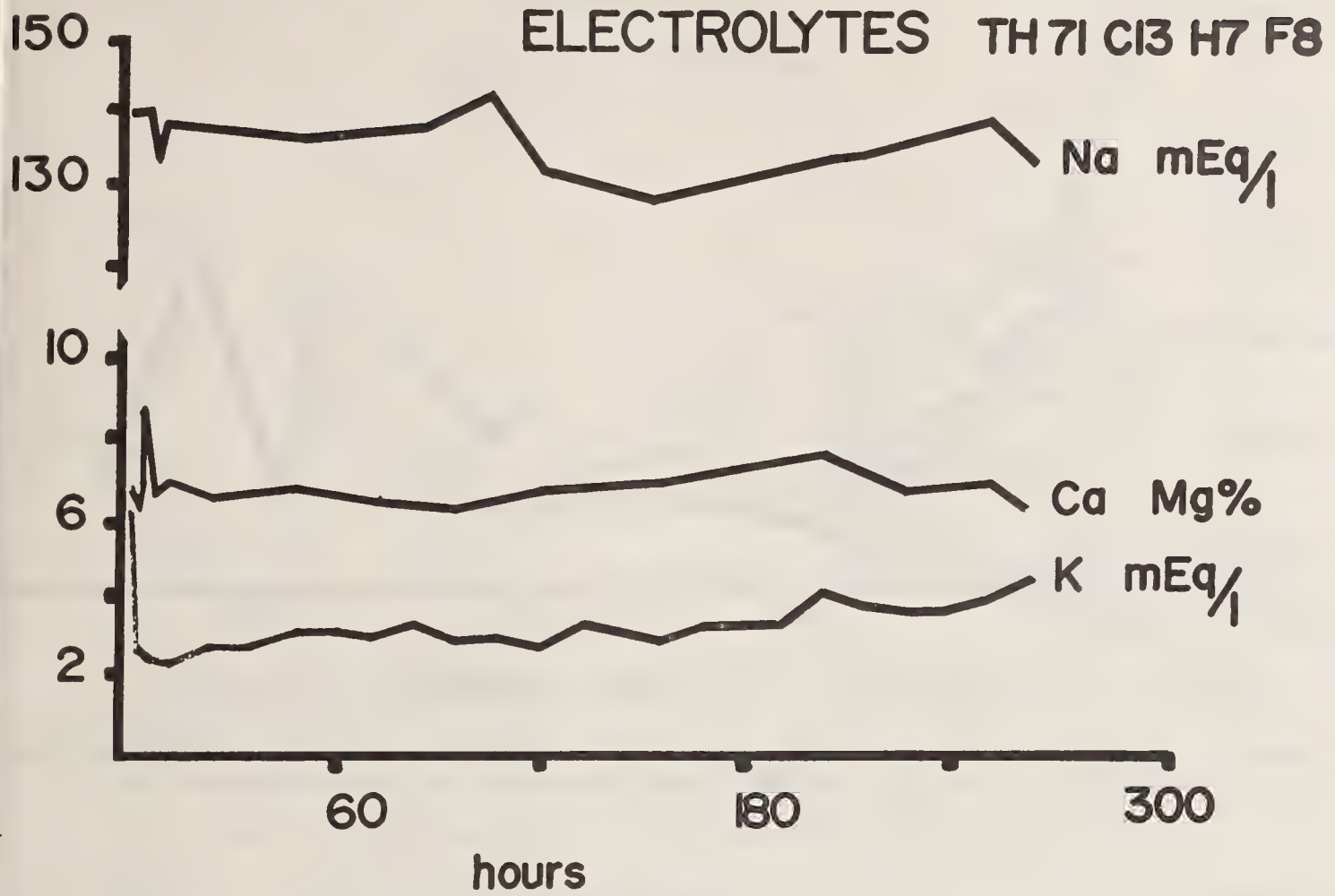


FIGURE 9.—Plasma electrolytes in a long-surviving calf with an artificial heart. Sodium remains close to control values while calcium and potassium tend to decrease in concentration with the initiation of pumping and remain lower than control values. The initial increase in potassium is due to administered potassium which is goven routinely postoperatively and with the use of diuretics.

The blood urea nitrogen for a long-surviving calf is shown in Figure 16.

POSTOPERATIVE CARE PROCEDURE

After surgery, two people remain with the calf at all times to monitor the clinical condition of the animal and to see that all of the recording and support equipment is operating properly. A fluid balance sheet is maintained on the animal and intravenous fluids and analgesics are given as required. Each 12 hours, 10 million units of Penicillin and 0.5 grams of Streptomycin are administered. Diuretics are administered as necessary to maintain urine output. The animal is allowed to eat and drink *ad libitum*. The animals are maintained on a Bird respirator or nasal oxygen to maintain an arterial oxygen saturation of at least 90%. Blood from donor calves is given as required to maintain the hematocrit about 25–35%, All urine produced by the animal is saved for analysis.

AUTOPSY

At termination, the animal is given 20,000 units of heparin to prevent post-mortem clotting. The animal is suspended in a physiological position by bone pins inserted through the vertebral spines (Figure 17). The brain is removed and sectioned. The right and left chest walls are removed leaving the sternum intact. If there is hydrothorax, a sample is obtained for analysis and the volume measured. The exposed lungs can now be inspected and inflated by an endotracheal tube. The lungs are then removed and sectioned for macroscopic examination. The

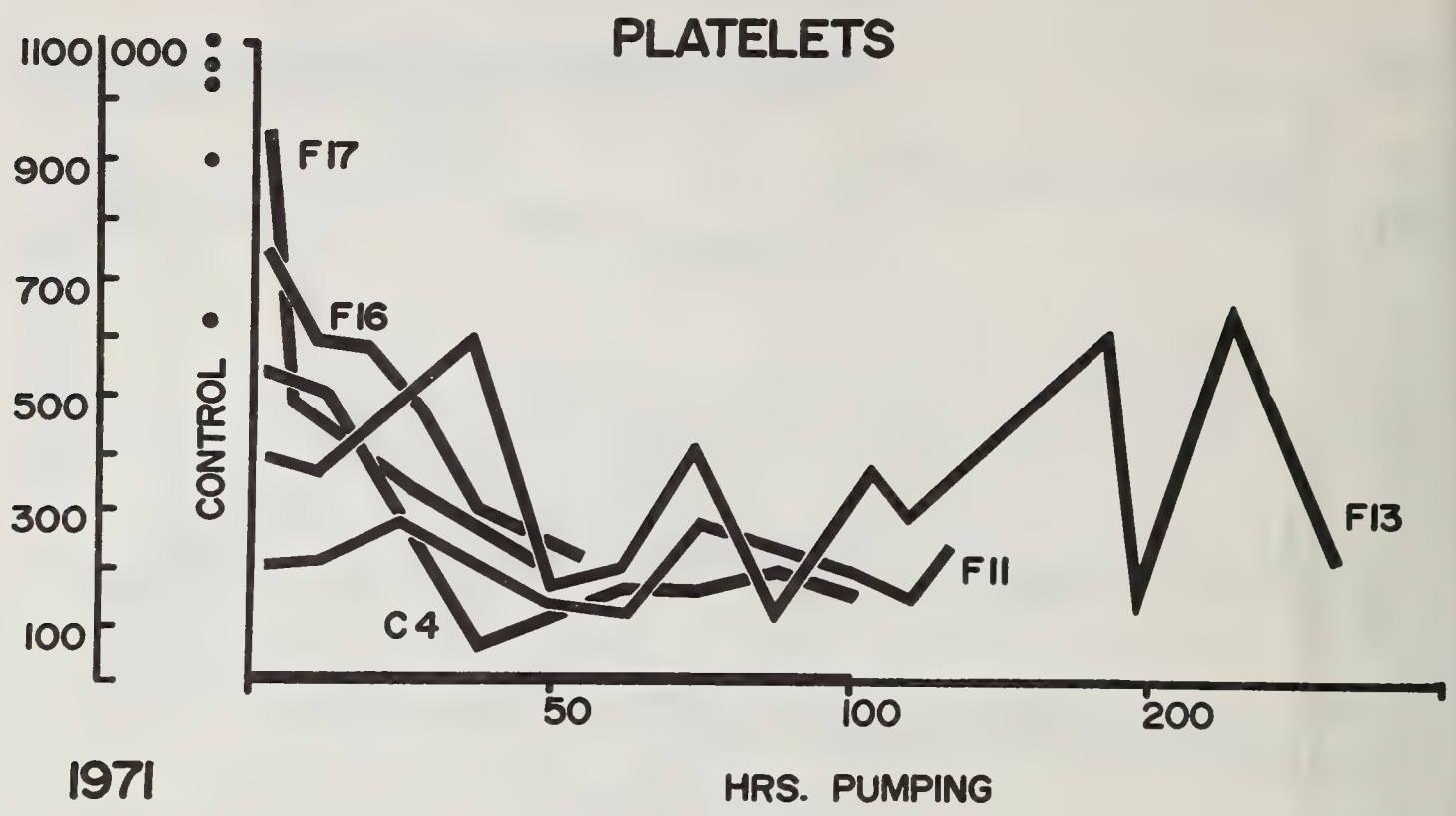


FIGURE 10.—Composite of a decreasing platelet count in five calves with time of pumping of the artificial heart. The coagulation system seems to be in a state of compensation with no hemorrhage occurring.

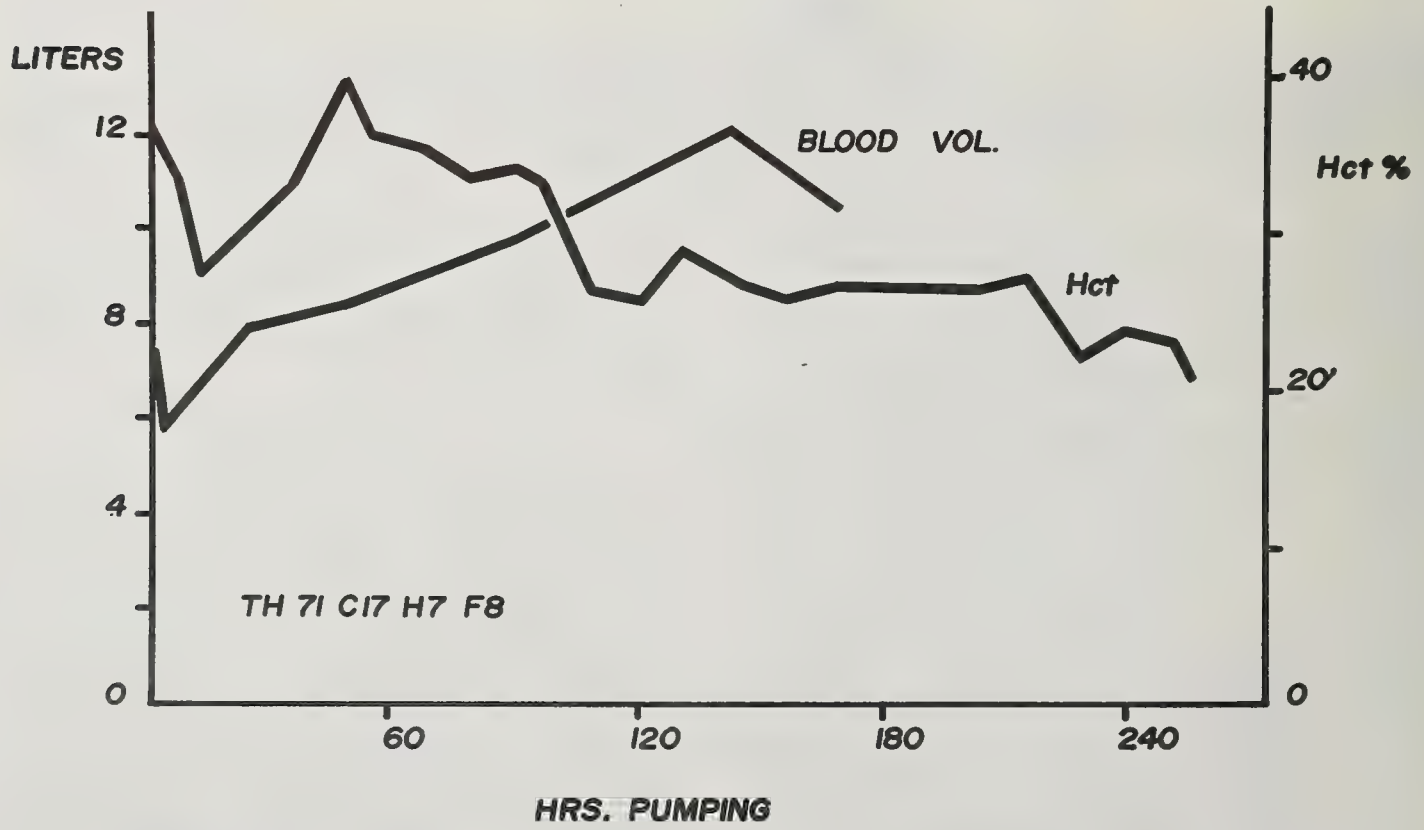


FIGURE 11.—Blood volume and hematocrit in a long surviving calf. Blood transfusions are given as required during pumping of the artificial heart.

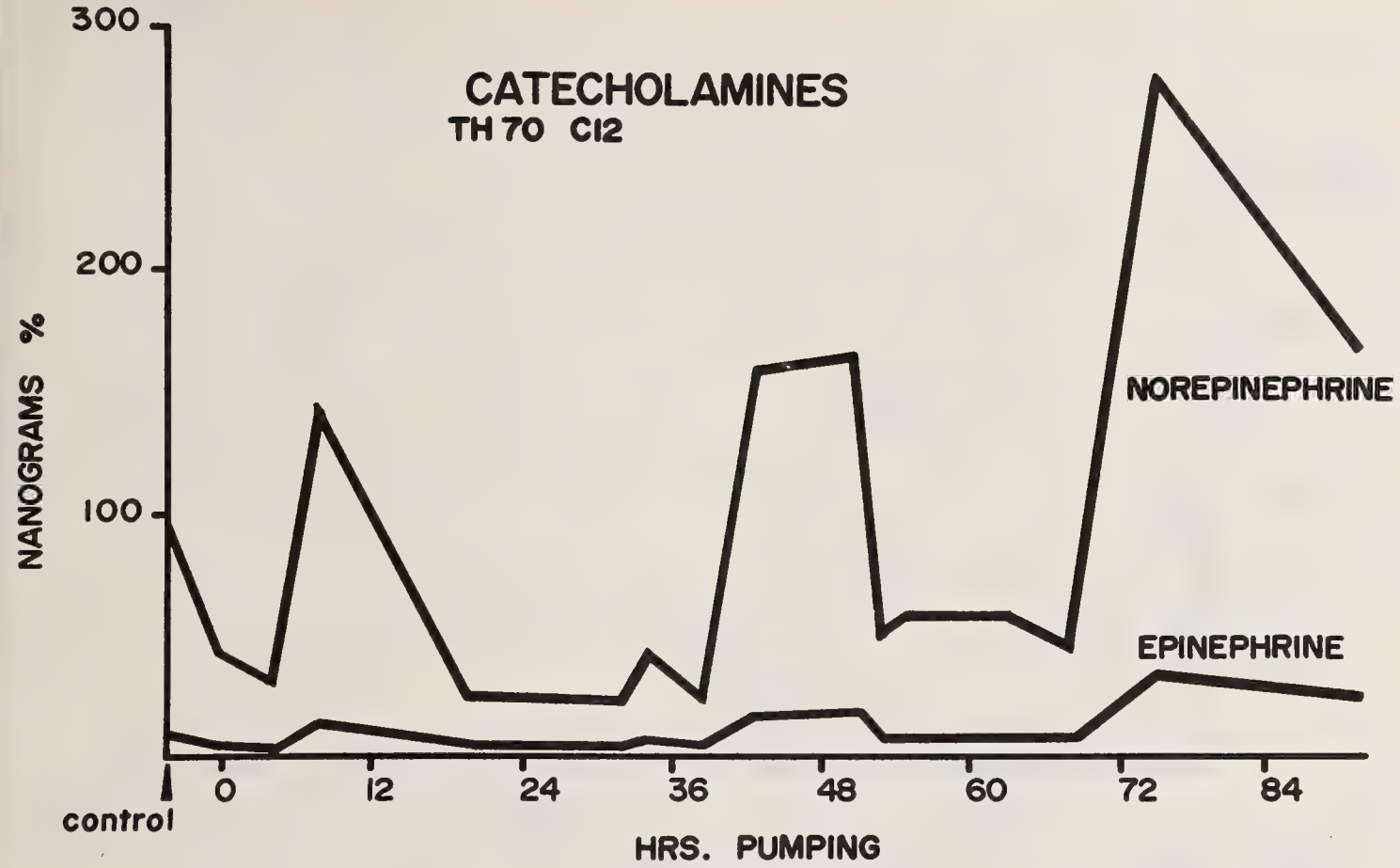


FIGURE 12.—Plasma epinephrine and norepinephrine in a calf with a total artificial heart.

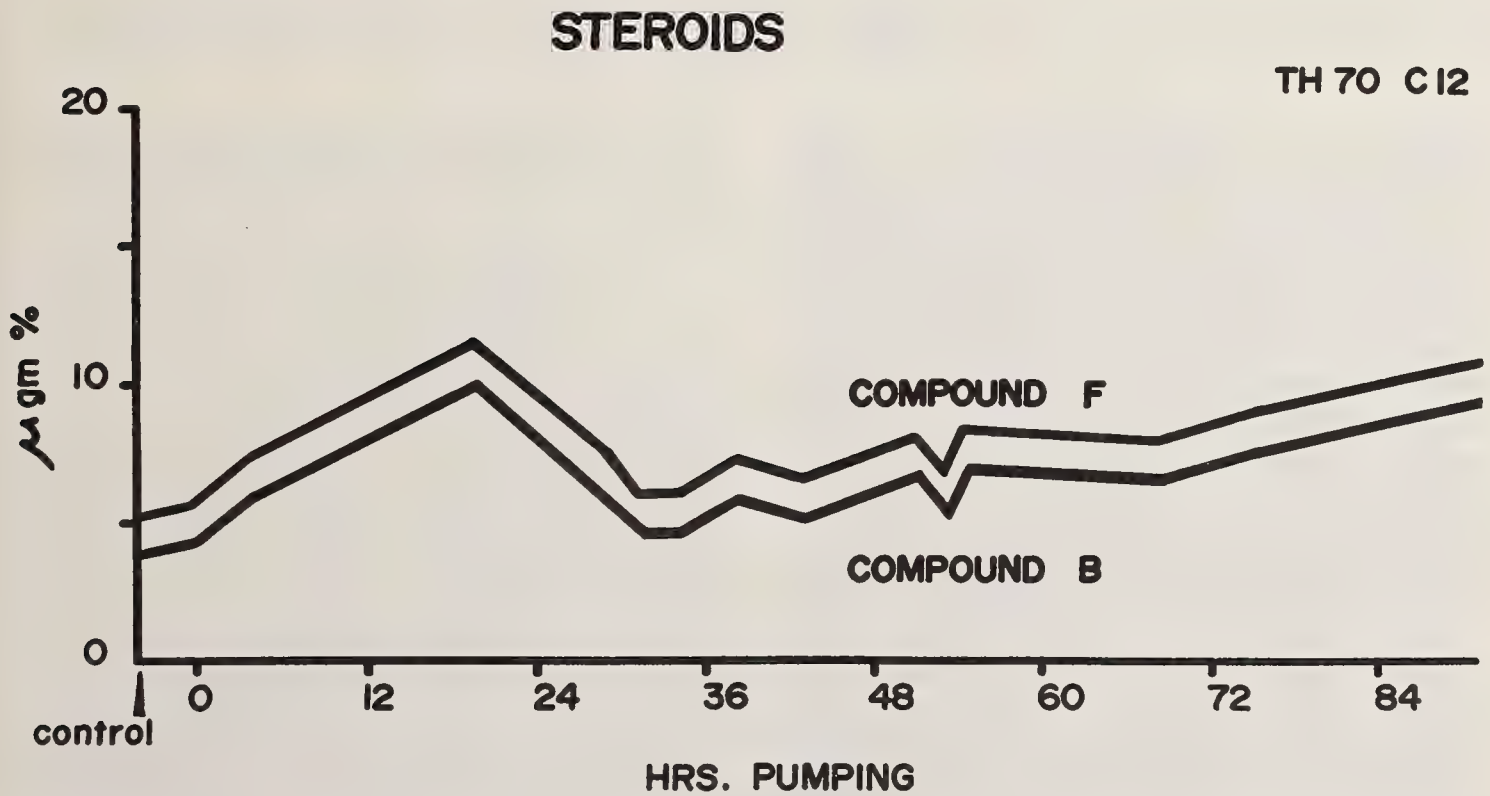


FIGURE 13.—Plasma steroids measured in the same calf (TH70 C12) as the plasma catecholamines were determined. (Figure 12).

TH 71 C13 H7 F8

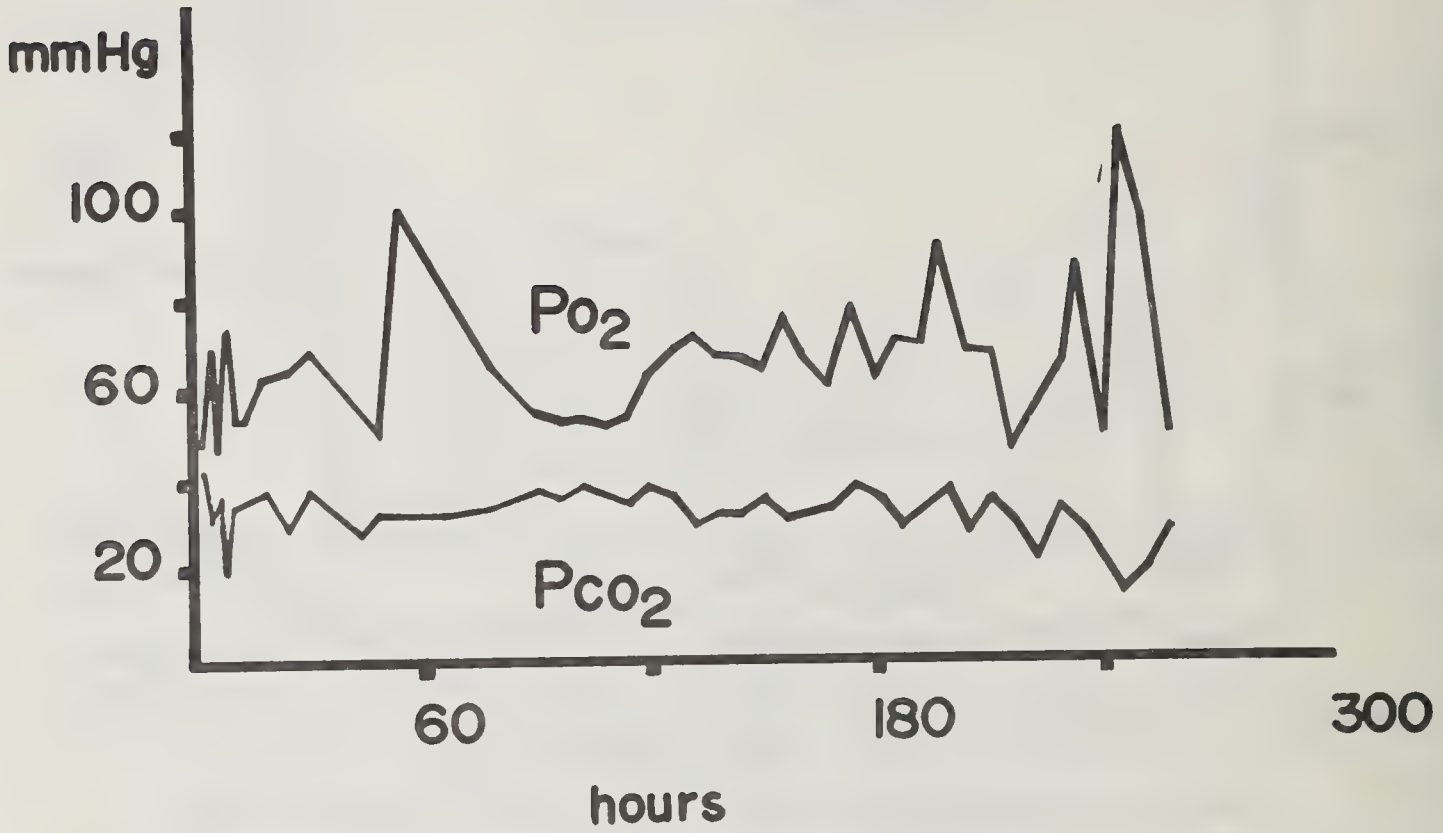


FIGURE 14.—Arterial pO_2 and pCO_2 in a long-surviving calf with an artificial heart. This calf required no respiratory support for eight days.

artificial heart is inspected while it is still in position to see how well it fits and to determine if vessels have been kinked and what corrections in the shape of the heart, if any, must be made. The artificial heart is then removed and inspected grossly and the diaphragm cut for electronmicroscopy. The abdomen is opened and the liver, spleen, kidneys and a section of the small intestine are removed for macroscopic examination. If ascites is present, a sample is taken for analysis and the total volume measured. When bacteremia is suspected, tissues from all organs are removed as sterile as possible for tissue culture. The volume and condition of fluid in the urinary bladder is determined. After sectioning and gross examination, samples of the lung, liver, kidney, spleen and brain are sent to a veterinary pathologist (Dr. Kent R. Van Kampen, Veterinary Pathology Service, 166 East 5900 South, Murray, Utah) for microscopic examination and report.

DURATION AND QUALITY OF LIFE IN LONG-SURVIVING CALVES

In ten calves with total artificial hearts surviving beyond the immediate effect of surgery (i.e., calves surviving beyond 40 hours), the average survival time was 94.1 hours (range 41.4 to 260 hours). Six calves regularly supported themselves with pumping of the artificial heart by spontaneously standing and lying down. The systemic arterial blood pressure remained stable at a peak systolic pressure of approximately 150 mmHg as illustrated in a long-surviving calf (Figure 6). Venous pressure (Figure 7, F–13) and blood volume (Figure 11), however, continually in-

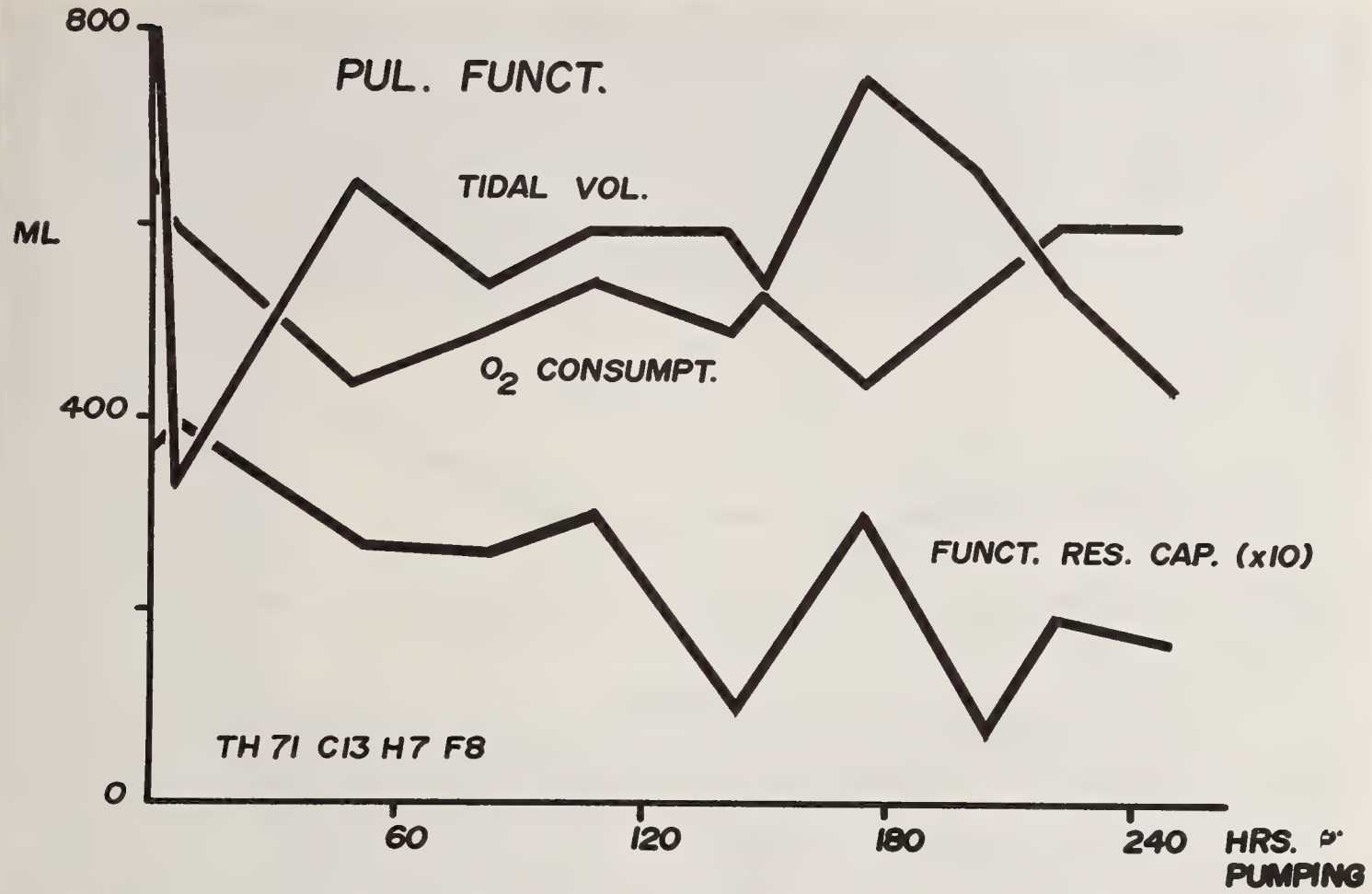


FIGURE 15.—Tidal volume, O_2 consumption and functional residual volume in a calf with an artificial heart.

creased with the time of pumping. This is currently one of the major problem areas to be solved in artificial heart research, namely, to reduce the venous inflow pressure and still maintain an adequate cardiac output. Respiratory function was variable as some calves required nasal O_2 or respirator support while the long-surviving calf (Figure 14 and 15) required no respiratory support for eight days, but did exhibit a gradually decreasing functional residual volume (from 4 liters to below 2 liters). Varying degrees of atelectasis were found in all calves at autopsy. All calves produced urine either spontaneously or by stimulation with diuretics. In calves with high hemolysis (i.e., artificial hearts with rough dacron surfaces), plasma hemoglobin usually ranged above 30 mg% and there was hemoglobinuria. Terminally, this was manifested by an increasing BUN (Figure 16) and red cell casts, tubular necrosis and infarction, and areas of congestion in the kidney upon microscopic examination. In calves with smooth artificial heart surfaces, hemolysis was low, usually below 10 mg% however, there was greater evidence of embolization in the kidneys, brain and lungs, suggesting that the fibril surface, although it caused greater hemolysis, provided a reticulum dacron structure for a pseudo-intima to form. Two calves were terminated because of obvious sepsis. In one calf, (260 hour survival) the intima of the artificial heart was lined with a green (Pseudomonas cultured) membrane.

Other pathological findings at autopsy included congestion and/or central lobular necrosis of the liver and an average 1.2 liters of ascites most likely from the acute high central venous pressure.

In spite of these drastic physiological changes, the calves were usually responsive to

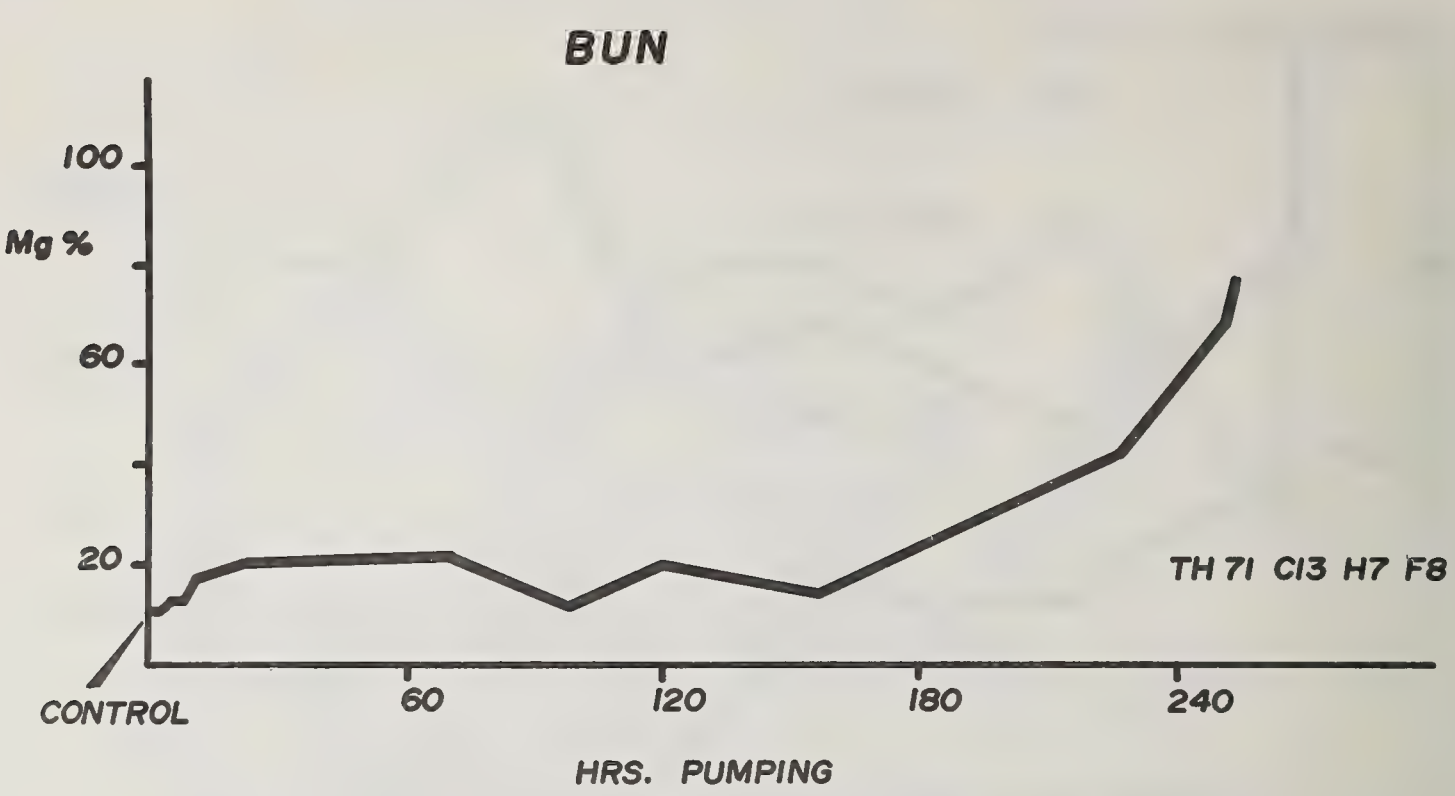


FIGURE 16.—Blood urea nitrogen in a long-surviving calf with a rough surfaced artificial heart. This calf had significant blood damage and septicemia at the termination of the experiment.

their surroundings, at prepared food, drank water, produced urine and passed stools. It is currently believed that the coagulation system is in a state of compensation since the platelet count gradually decreases with the time of pumping (Figure 10) without evidence of hemorrhaging.

Survival doesn't seem to be limited because of catecholamine depletion since measurable plasma values have been obtained throughout the duration of pumping (Figure 12).

CONCLUSION

The development of systematic and standardized procedures for preconditioning of calves, implantation of the artificial heart and postoperative care have been an attempt to minimize uncontrollable variables and more accurately assess the effects of the artificial heart. These effects have resulted in: 1) a standardized research calf and a decreased probability of having a diseased calf for implantation of the artificial heart; 2) capability of separating the blood damage effects (e.g., red cell damage) of cardiopulmonary bypass from those of the artificial heart by using deep hypothermia; and 3) obtaining calves surviving beyond the immediate effects of thoracic surgery resulting in new problem areas (e.g., high inflow pressures) and new design criteria for future artificial hearts.

REFERENCES

1. KWAN-GETT, C. S., ZWART, H. H. J., KRALIOS, A. C., KESSLER, T., BACKMAN, K. and KOLFF, W. J. A. Prosthetic Heart with Hemispherical Ventricles Designed for Lowered Hemolytic Action. Trans. Amer. Soc. Artif. Int. Organs, 16:409–415, 1970.
2. KAWAI, J., DONOVAN, F., PETERS, J., ZWART, H., KESSLER, T., EASTWOOD, N. and KOLFF, W. J. Physiological Effects of Artificial Heart in Calves Surviving up to Ten Days. American Physiological Society Meeting, Lawrence, Kansas, Aug. 16–19, 1971, and published in The Physiologist, Vol. 14, No. 3, p. 170, Aug. 1971.
3. KAWAI, J., PETERS, J. L., DONOVAN, F. M., JR., ZWART, H. H. J., HERSHGOLD, E. J. and KOLFF, W. J. Successful Use of Deep Hypothermia for Implantation of the Artificial Heart in Calves. Circulation Supplement II, Vol. XLII and XLIV, No. 695, Oct. 1971.

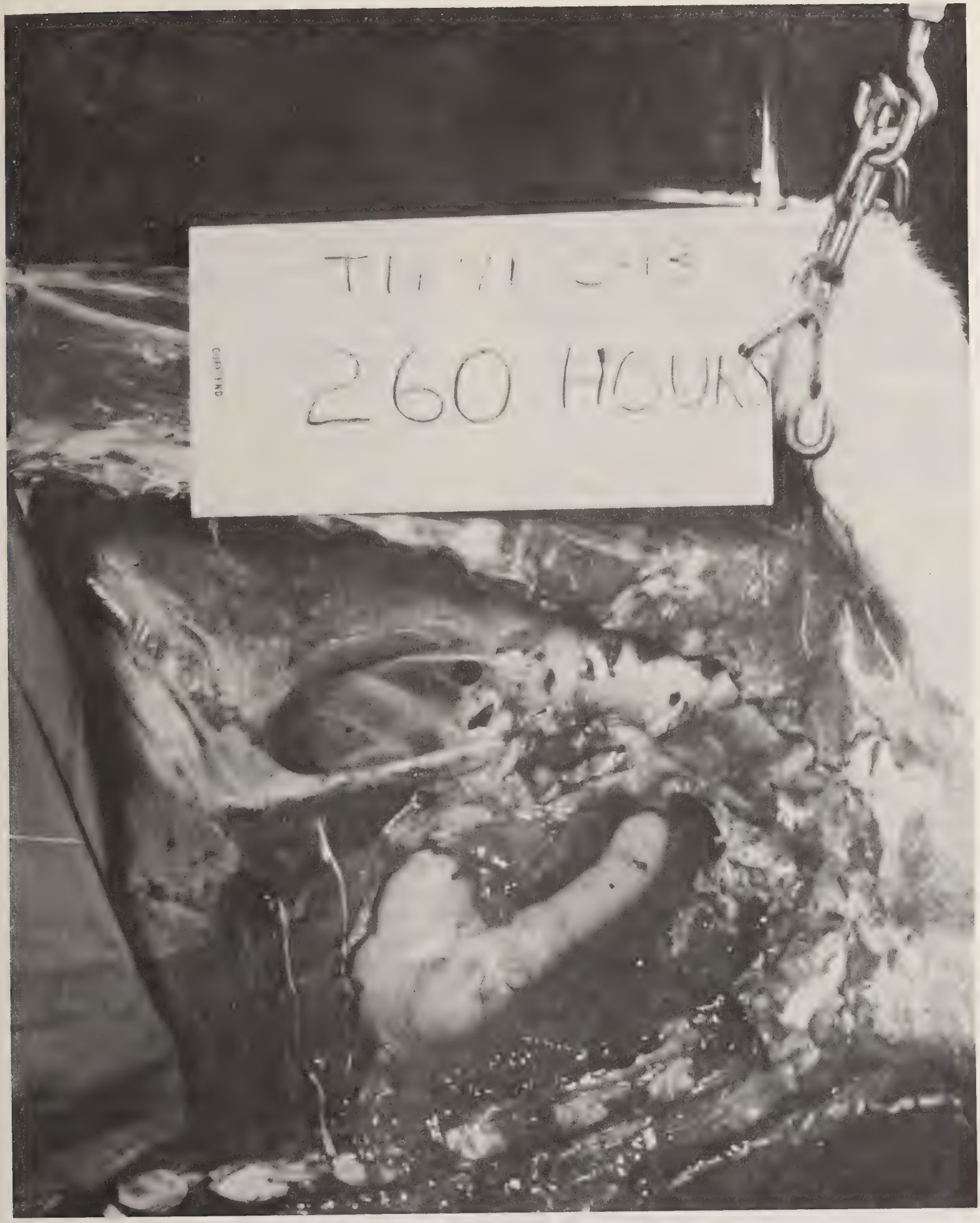


FIGURE 17.—Suspended calf at autopsy with the right rib cage and lung removed, illustrating the fit of the right ventricle, right atrial and pulmonary artery connections. The diaphragm is on the left side of the figure.

4. Kwan-Gett, C. S., Backman, D. K., Donovan, F. M., Eastwood, N., Foote, J. L., Kawai, J., Kessler, T. R., Kralios, A. C., Peters, J. L., Van Kampen, K., Wong, H. K., Zwart, H. H. J. and Kolff, W. J. Artificial Heart with Hemispherical Ventricles II and Disseminated Intravascular Coagulation. Trans. Amer. Soc. Artif. Int. Organs, 17:474–481, 1971.
5. Hershgold, E. J., Kwan-Gett, C., Rowley, K. M. and Kolff, W. J. The Effects of the Total Artificial Heart on Hemostasis and Coagulation. Clinical Research, 20:185, February 1972.
6. Kwan-Gett, C. S., Kawai, J., Peters, J. L., Donovan, F. M. and Kolff, W. J. Consumption Coagulopathy as a Limiting Factor for the Total Prosthetic Heart. American College of Cardiology Meeting, February, 1971.
7. DeVries, W. C., Kwan-Gett, C. S. and Kolff, W. J. Consumptive Coagulopathy, Shock and the Artificial Heart. Trans. Amer. Soc. Artif. Int. Organs, 16:29–36, 1970.
8. Donovan, F. M., Jr., Peters, J. L., Kawai, J., Zwart, H. H. J., Van Kampen, K. R. and Kolff, W. J. Cardiopulmonary Effects of Total Artificial Heart in Calves Surviving up to Ten Days. Circulation Supplement II, Vol. XLIII and XLIV, No. 595, Oct. 1971.
9. Peters, J. L., Donovan, F. M., Zwart, H. H. J., Kawai, J., Van Kampen, K., Kwan-Gett, C. S. and Kolff, W. J. Physiological and Metabolic Changes in Calves Surviving Up to One Week with a Total Artificial Heart. American College of Chest Physicians Meeting, Philadelphia, Penn., 24–28, Oct. 1971, and Chest, Vol. 60, No. 3, Sept. 1971.

THE USE OF LARGE ANIMALS IN EXPERIMENTAL CARDIOVASCULAR SURGERY

J. N. Ross, Jr., J. M. Fuqua, Jr., B. S. Ruark, and J. H. Kennedy*

Seven species are compared in terms of their usefulness as experimental animals in a cardiovascular surgery program. The authors selected the calf since most of our work necessitated considerable handling of cardiovascular tissues, a tolerance to chronic transcutaneous instrumentation, a tolerance to cardiopulmonary bypass and a tolerance to chronic mechanical circulatory support or replacement devices. The necessity for preoperative conditioning is emphasized, as are the means of anesthetic and operative management. It was found that calves are relatively easy to manage postoperatively; however, this does not mean that one can neglect them after intensive surgical procedures. The primary difficulties encountered when using this species in an experimental cardiovascular laboratory include their susceptability to upper respiratory infections, the fact that they are not mature animals, the fact that their thorax does not conform to that of man or subhuman primates, and the fact that electrical defibrillation in larger animals is difficult with existing equipment.

INTRODUCTION**

Cardiovascular disease is the leading cause of death in the United States, and, in addition, 15,000,000 Americans aged 18 to 79 have heart disease.[1] These facts have given rise to a vigorous research effort to develop techniques for prevention, recognition and treatment of cardiovascular disease, as well as devices to assist or replace the failing circulation. To support this effort the larger laboratory mammals (i.e., subhuman primates, dogs, pigs, ponies, goats, sheep, and calves) have been used with increasing frequency primarily because of their size. Historically, several of these species have been intimately associated with the development of man and medicine, beginning with the domestication of the pig and dog over 16,000 years ago. The early development of experimental medicine is generally associated with the Greeks. Galen used several species, especially the Barbary ape, relating structure to function within the mammalian species, as he observed and dissected animals and cadavers from the arena, and he is credited with being the father of comparative anatomy and experimental medicine.[2]

This paper summarizes our experience with 380 calves in an experimental cardiovascular surgical laboratory and discusses the suitability of bovines relative to the other species listed above.

EXPERIMENTAL OBSERVATIONS

In the past five years over 380 calves have been used in our surgical laboratories for a variety of experiments including: the evaluation of prosthetic heart valves; the evaluation of the Baylor-Rice paracorporeal left ventricular bypass in normal animals and in animals with induced cardiac failure;[3,4] the evaluation of other means of cardiac assistance such as intra-aortic balloon pumping and counterpulsation; for studies involving chronic implantation of instrumentation for hemodynamic measurements; and for evaluation of the Baylor-Rice total orthotopic cardiac prosthesis.

Preoperative Care

Problems were initially encountered in procurement. Calves purchased at a local sale barn proved to be completely unsatisfactory for experimental surgery since 50% of these animals had upper and/or lower respiratory disease, parasitism or anemia. In addition, the animals were intractable. The seasonal unavailability of calves of the desired sex and weight range, particularly during the winter months was another deterrent to this form of purchase. Female calves are preferred since catheterization

*Baylor College of Medicine, Houston, Texas

**Taub Laboratories for Mechanical Circulatory Support, Cora and Webb Madding Department of Surgery, Baylor College of Medicine, Houston, Texas 77025. Supported in part by HEW NIH grants HL-13330, HE-05435, and Special Fellowship 1F03 GM 45166 (Ross).

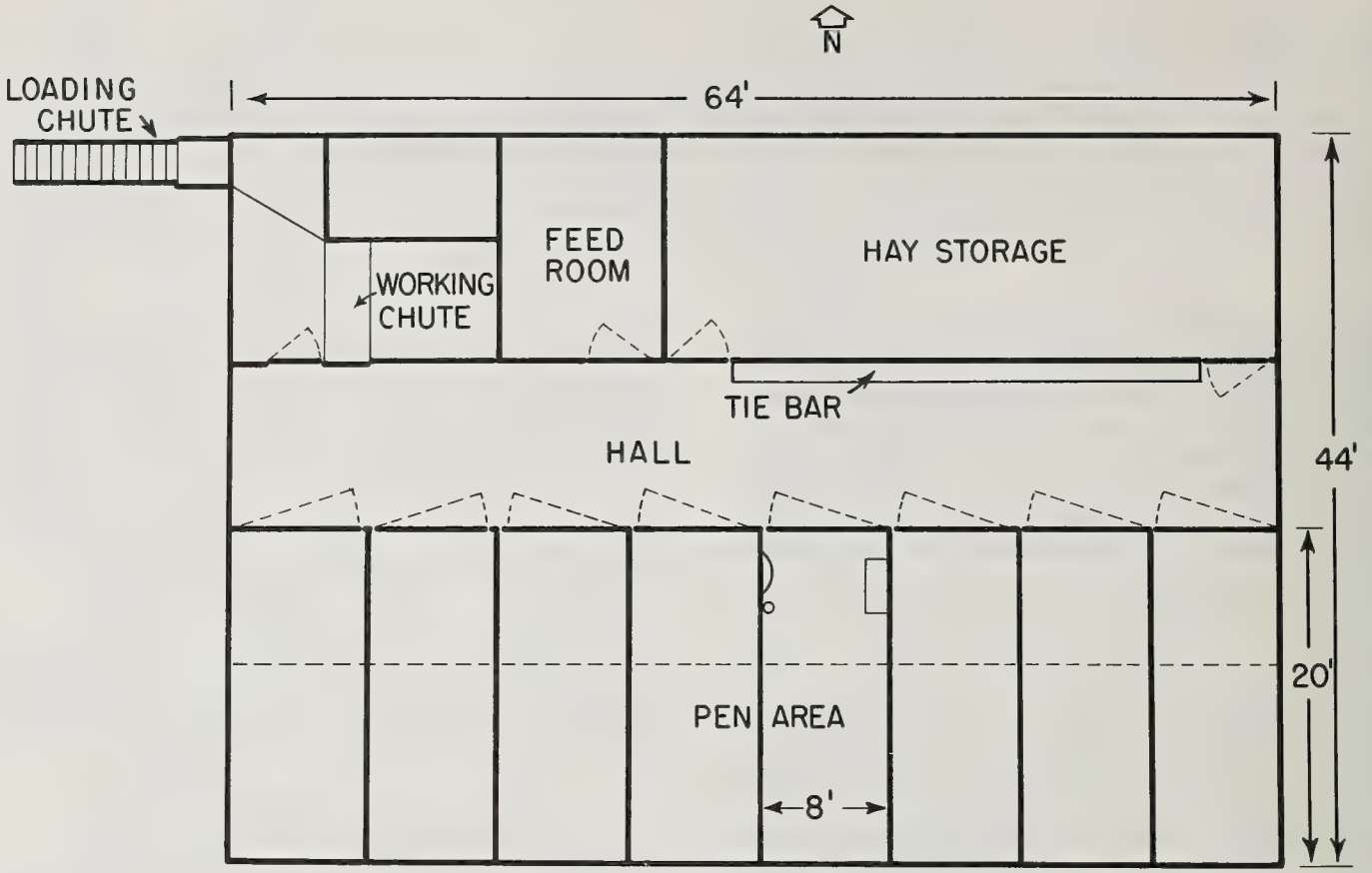


FIGURE 1.—Line drawing of floor plan for calf preoperative care building. The floor is rough concrete. Housing is the indoor-outdoor type with roof extending to dotted line through pen area.

of the bladder is routinely employed in our experiments. Consequently, local ranchers were contacted and arrangements were made for the direct purchase of "apparently healthy" calves. A facility was constructed (Figure 1) in conjunction with the Texas Department of Corrections, Wynne Unit, Huntsville, Texas, for preconditioning the animals 2 to 4 weeks prior to surgery.

Upon admission to this facility the animals are examined, haltered, identified, sprayed for ectoparasites, wormed, vaccinated for respiratory viruses and treated for any other obvious disease. During the conditioning period fecal samples and blood specimens are taken to establish baseline data and any sick animals are appropriately treated. Particular attention is paid to nutrition during this time and the animals are individually weaned to a ration of pelleted alfalfa and grain.[5] They are handled daily and consequently they are extremely docile when brought to the medical facility several days prior to surgery. During the period prior to surgery they are individually stalled and observed, and become accustomed to the laboratory (Figures 2 and 3).

Anesthesia

Prior to surgery, food and water is withheld for 12–18 hours. Longer periods of fasting in this species may add undue stress and lead to dehydration and metabolic acidosis.[6] Pelleted alfalfa in the ration may help to safely shorten fasting without fear of regurgitation and aspiration during anesthesia.

Anesthesia is induced via face mask with 2% halothane vaporized into a partial rebreathing circle system by 50 to 70% nitrous oxide in oxygen at the level of 5 L/min. Respiration is manually assisted during the induction period using periodic "sighs". Pre-medication is not routinely given. Muscular relaxation is sufficient to permit tracheal intubation within 10 minutes.

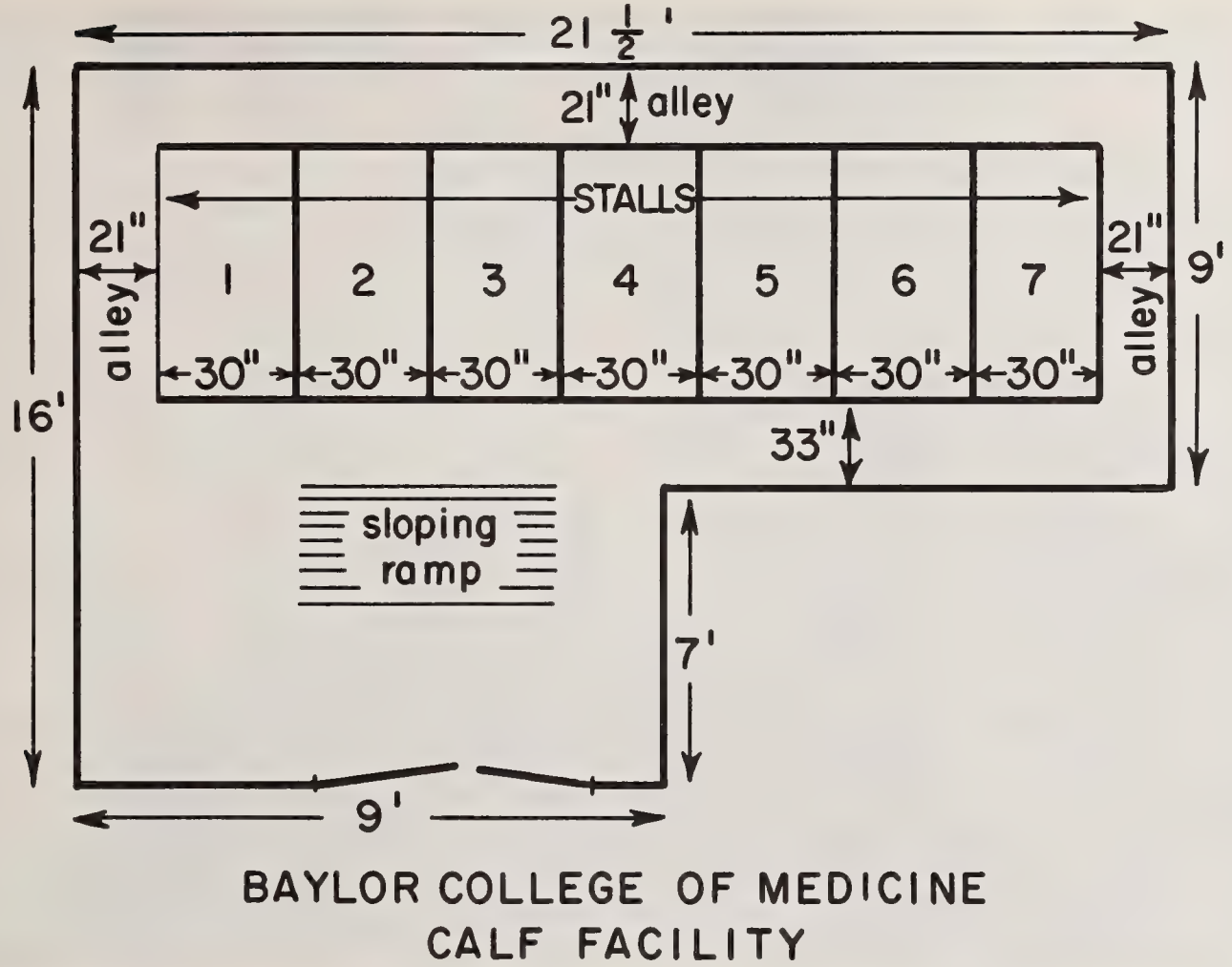


FIGURE 2.—Line drawing showing dimensions of facility used to accommodate calves in the immediate pre- and postoperative periods.

Following tracheal intubation, anesthesia is maintained with a mixture of 0.5 to 1.0% halothane in equal parts of nitrous oxide and oxygen. A peroral stomach tube is inserted into the rumen to prevent gastric tympany from developing during the operative procedure. In artificial heart implantation procedures a tracheostomy is performed facilitating prolonged postoperative ventilatory assistance if necessary.

Once the chest is open, ventilation is mechanically maintained by the use of a Bird 4/8 combination anesthesia assister-controller and/or by manual means. It is necessary to use inspiratory pressures of 28–33 cm H_2O to prevent alveolar collapse in this species.[7] The effectiveness of anesthetic management is periodically assessed by the analysis of arterial blood samples (pH, pO_2, and pCO_2). During most surgical procedures, the animals are maintained in light surgical anesthesia. Muscle relaxation, when necessary, is obtained with the use of intravenous succinylcholine chloride.

Thoracic Surgery and Cardiopulmonary Bypass (CPBP)

In our experience, the calf is particularly suitable for CPBP. Intravascular cannulation can be accomplished via a variety of surgical exposures. However, median sternotomies are avoided whenever possible because of complications which arise when placing the calf in dorsal recumbancy. These complications include ventilation/perfusion imbalances in the lung and the rapid development of ruminal tympany (bloat). Increased pressure of abdominal viscera on great vessels causes obstruction of venous re-



FIGURE 3.—Photograph of portion of the facility in Figure 2. Partitions are aluminum and easily removable. The elevated false floors facilitate cleaning and keep the animals dry.

turn and a consequent reduction of cardiac output.[6,8,9,10] In addition, since the calf naturally lies on its sternum, postoperative infection and pain are more of a problem after sternotomy.

Normothermic hemodilution using a disposable bubble dispersion oxygenator (Travenol 6 LF) primed with 3 liters of 5% dextrose in distilled water and roller pumps (Sarns 3500) is routinely employed for CPBP in our laboratories. During CPBP the lungs are statically inflated at 10 cm of water pressure and further inflated to 25–30 cm of water pressure every 5 minutes. A single cannula is inserted via the right atrial appendage into the right ventricle for venous drainage when using a left thorocotomy approach. Arterial perfusion is via a cannula inserted into the descending thoracic aorta. This technique has worked particularly well for the insertion of mitral valve prostheses.

During the insertion of the Baylor-Rice total orthotopic cardiac prosthesis, a right thorocotomy using a 5th rib resection is used and both vena cavae are cannulated via the right atrium for venous drainage. Arterial perfusion is again via a single cannula in the descending thoracic aorta which is mobilized within the dorsal mediastinum. Cardiopulmonary bypass flows of 40 to 60 cc/kg/min are routinely obtained by this method using approximately 54 cm of gravity drainage for venous return to the heart lung machine. Although the values are low when compared to other species, in our experience these flows are well tolerated in the calf for periods of up to 1½ hours.

Other uses in experimental cardiovascular physiology for which the calf has been found particularly suitable include the application of chronic instrumentation to the heart and great

vessels. The epicardial vessels of the calf are easily accessible and of good size; i.e., the main left coronary artery is suitable for the application of flow probes in the 5 to 7 mm range in a 100 kg animal. Anatomic peculiarities of the calf include the presence of the hemiazygous vein which drains into the coronary sinus. The hemiazygous vein can be ligated without complication and is a useful route for catheterizing the coronary sinus to obtain blood samples. The aortic arch of the calf may present a problem since the ascending aorta is extremely short (about 1.2–2.5 cm in a 100 kg animal) giving rise to a single brachiocephalic trunk. However, flow transducers can be placed around the ascending aorta and pulmonary artery without difficulty. We have found that 22 to 26 mm probes fit well on these vessels in the 100 kg calf.

Electrical defibrillation of the heart is frequently not possible with commerically available equipment because of the animals' extremely large cardiac mass. However, a combination of pharmacological and electrical means has been successfully employed in a number of cases in our laboratory. When defibrillation is necessary, the heart is arrested by the rapid left intraventricular injection of potassium chloride (20–80 mEq) during manual massage until fibrillation ceases. Normal acid-base balance is restored by the intravenous injection of sodium bicarbonate in amounts necessary to correct the base deficit. While continuing cardiac massage, an intravenous infusion of 1 g of calcium chloride is begun slowly and continued to effect. Full cardiac contraction is restored by these methods alone in approximately 30% of all cases. In others which return to fibrillation, electrical defibrillation has been occasionally successful.

Postoperative Management

Postoperative management of the calf necessarily varies with the experimental procedure. In general, the animals are placed in sternal recumbancy as soon as possible, preferably within 8 hours after the operation. The calf tolerates externalized leads, wires, catheters and cardiac assist devices relatively well for long periods of time. Chronic monitoring in the facility (Figure 3) has proven adequate for observing animals postoperatively for 3–5 weeks. After this time, it is desirable to provide the animals with more freedom since their ability to exercise in there quarters is extremely limited. This has been a problem in our laboratory, and when possible during long-term experiments, the animals are returned to the preoperative conditioning facility for exercise.

DISCUSSION

Considerations other than size when selecting an experimental animal for any given cardiovascular surgical procedure include: anatomical and physiological peculiarities of the species; age and rate of growth; local availability and cost; facilities and space required for husbandry and ease of handling; tolerance to anesthesia and surgical procedures; and spontaneous diseases frequently encountered in the species. While it is not the purpose of this paper to fully discuss each of these topics, certain generalities are warranted regarding species selection for any given cardiovascular surgical procedure.

In Table I, seven species are rated in 11 categories relative to their comparative suitability for experimental cardiovascular surgery. It is based on the authors' experience with 380 calves, 160 sheep, 30 pigs, 20 goats and hundreds of dogs and on the experience of others as indicated in the references under each species.

Cost/availability is, of course, regional. The relative availability and the low cost of dogs and the relative high cost of primates of human size, at least in our area, has limited our own use of the latter. However, the increasing cost of institutionally bred and reared canines has caused us to reconsider this matter.

Husbandry refers to the relative ease of production, housing, and all phases of preoperative care and conditioning necessary to obtain a suitable animal for chronic experimental cardiovascular surgery. Most laboratories have the facilities to house and care for dogs and are familiar with their needs. Relatively few laboratories are equipped to handle the requirements

TABLE I.—*Considerations In Selecting Species for Experimental cardiovascular Surgery: Relative Comparison*

	Primate	Dog	Pig	Pony	Goat	Sheep	Calf
Cost/Availability	−	+++	++	++	++	++	++
Husbandry	±	+++	++	++	++	++	+
Size/Maturity	±	+++	+±	+++	+++	+++	−
Anesthesia	++	+++	+±	++	++	++	++
Thoracic Exposure	+++	+++	−	+	++	+	++
CV-Human Similarities	++++	++	+++	+±	+±	+±	+±
CPBP Tolerance	+++	+	−	?	+?	+	+++
CV-Tissue Tolerance	++	+	−	+++	++	+	+++
Postop Management	±	+±	±	±	+	+	++
Instrumentation Tolerance	−	+	+	+++	++	++	+++
Chronic MCA Tolerance	±	±	±	?	+	++	+++
References	11–13, 111–131	14–38, 95, 97, 98	39–48, 95, 96, 100	49–58, 99–105	10, 59, 62	61, 63–71, 101, 104	7–9, 72, 91, 92, 94, 102, 109, 110

− = Poor, ? = insufficient information, +(s) = degree of acceptability

of approximately 70 kg primates. While the other species are relatively easy to care for, special facilities are necessary to meet the husbandry requirements of each.

The importance of establishing rigorous procedures and adequate facilities for the procurement and preoperative conditioning of animals prior to attempting extensive experimental cardiovascular surgery cannot be overemphasized. It is imperative in most experiments that sound, healthy animals be used in order to minimize the variability of the experimental results and to maximize tolerance to anesthesia and surgical trauma. Spontaneous disease problems occur in all species of laboratory animals and necessitate constant surveillance. For example, screening for heartworm disease is necessary in randomly-procured dogs, particularly in the coastal areas where *Dirofilaria immitis* is endemic. The frequency of this disease is a deterrent to the use of the canine in many cardiovascular studies. The problems of respiratory disease in calves has been noted by many investigators.[74–77,92] Our laboratory has largely solved this problem by culling and conditioning these animals as previously described.

If animals of approximately 70 kg are required, their maturity at this weight may be an important consideration. Relative to the other species, the calf is less mature for a given weight and gains approximately 1 lb per day. This can be a disadvantage in studies involving long term observation of implanted prostheses, e.g., following prosthetic valve implantation, relative valvular stenosis results.[3]

While a number of difficulties have been encountered in the anesthetic management of each of the above sub-human mammals, most problems can and have been overcome with experience. Anesthetic induction has been a problem in the pig because of the small size of the accessible vessels.[39] In addition, perilaryngeal anatomy of pigs has rendered tracheal intubation difficult in the hands of some investigators.[39] The anesthetic management of calves has also been variably reported.[7,8,86,93,94] While some investigators have had good success with barbiturate anesthesia in calves;[86,93,94] others,[7] including the authors, have noted that the use of barbiturates is accompanied by prolonged recovery periods. Inhalation anesthesia, particularly with halothane and methoxyflurane, has more recently been used in most of the species.[11,16,42,49,59,64,86] Incorporating nitrous oxide into the gaseous mixture allows one to reduce the concentration of the primary anesthetic agent.

The depressing effects of halothane on myocardial function have been documented in a number of species.[42] Prolonged pre-ejection periods in calves have been observed by the authors and are being reported elsewhere, further supporting the depressing effects of halothane on myocardial contractility.

The relative ease of gaining access to the chest through fat, muscle and ribs, as well as the relative accessibility of the heart and great vessels once the thorax is entered, is of importance when considering an animal species for experimental surgery. The pig has a par-

ticularly fat, thick and inelastic chest wall and this, coupled with the relative smallness of the thoracic cavity of the pig in relation to the overall size of the animal, makes thoracic exposure in this species difficult.[44] The pony's chest wall is relatively thick and may present some difficulty with rib retraction. Thoracic exposure presents no particular difficulties in the primate, dog or ruminant; however, among the latter, the authors have found the calf superior to both the goat and sheep.

The obvious advantage of the larger primates is their anatomic similarity to man. Blood groups in *Papio* species[11] and clotting factors in non-human primates in general[12] are reported to be similar to man. The pig has been frequently suggested as a laboratory animal for cardiovascular research largely because its coronary blood supply is very similar to the human's in anatomy and distribution.[39,41,96] Whereas all of these species are relatively similar hemodynamically, differences exist in terms of the distribution of Purkinje fibers and ventricular activation process,[97–101] and in the anatomy of the great vessels—notably the short aortic arch giving rise to a single brachiocephalic trunk, and the persistent vena hemiazygous in ruminants.[102]

The pig is not well suited for chronic studies necessitating cardiopulmonary bypass. Our experience with the pig has been similar to others in that total body perfusion has been consistently fatal within 8 hours after CPBP operations in this species.[43,44] Their poor tolerance to CPBP is characterized by rapid and progressive right heart distension associated with postperfusion pulmonary insufficiency similar to that described in man.[103] The pig may be a promising model for the study of this syndrome. The primate[11,13] and calf[85,87,88] tolerate CPBP well, and are preferred over the goat, sheep or dog for this purpose. The authors have no experience with CPBP in the pony or goat and no literature was found regarding total body perfusion in these species.

The friability of the cardiovascular tissues in the pig,[39,44] sheep,[64,104] and dog,[19,32,33] makes these species less suitable than the calf and pony in experimental cardiovascular surgical procedures.

Postoperative management of primates can be particularly difficult because of their mobility and manual dexterity, and this may be a deterrent to their use in studies necessitating chronic hard wire instrumentation and/or mechanical circulatory assistance (MCA). Similarly, dogs are basically undesirable for experiments requiring chronic transcutaneous conduits due to their tendency to bite, chew, scratch and in general disrupt external leads or assist devices. Considerable difficulty may be encountered in managing the pony in the immediate postoperative period of anesthetic recovery. All species present postoperative problems. In our experience, the calf presents fewer difficulties than any of the other species.

The calf and pony are relatively easy to manage and seem to tolerate chronic instrumentation well.[3,4,56] The authors find the pig, primate, and to some extent the dog, to be relatively unmanageable in studies involving prolonged mechanical circulatory assistance and instrumentation.

The equine species has historically contributed much to our knowledge of the cardiovascular system and circulation,[105–108] but it has been only recently that the pony has been adapted for routine use in a laboratory setting.[53–55,57,58] In general, the pony's potential as an experimental animal for cardiovascular surgery is appealing, but further studies are needed to define its tolerance to CPBP and prolonged mechanical circulatory assistance or replacement. The calf has been frequently used and has survived longer than any of the other species during prolonged mechanical circulatory assistance and replacement.[8,93,94,109,110]

Unquestionably, dogs have been used more than the other experimental animals, and an abundance of well documented surgical and physiological data has accumulated to serve as a firm basis for comparative investigation. Because of the difficulties encountered with cardiopulmonary bypass, transcutaneous instrumentation and restraint, availability in large size, tolerance to chronically applied circulatory assist methods or devices and spontaneous diseases such as heartworm infestation and homologous blood reactions,[28,29] the use of other species seems appropriate. To date, the calf

has been most useful in our laboratory because of its tolerance to CPBP and chronic mechanical circulatory assist/replacement devices.

SUMMARY

Seven species have been compared in terms of their usefulness as experimental animals in a cardiovascular surgery program. The authors selected the calf since most of our work necessitated considerable handling of cardiovascular tissues, a tolerance to chronic transcutaneous instrumentation, a tolerance to cardiopulmonary bypass and a tolerance to chronic mechanical circulatory support or replacement devices. The necessity for preoperative conditioning was emphasized, as were the means of anesthetic and operative management. It was found that the calf is relatively easy to manage postoperatively; however, this does not mean that one can neglect them after intensive surgical procedures. The primary difficulties encountered when using this species in an experimental cardiovascular laboratory include their susceptability to upper respiratory infections, the fact that they are not mature animals, the fact that their thorax does not conform to that of man or sub-human primates, and the fact that electrical defibrillation in larger animals is difficult with existing equipment.

REFERENCES

1. Hastings, F. W. and Harmison, L. T. Proceedings of the Artificial Heart Program Conference, HEW-USPHS-NIH, Washington, D.C., 1. 1, 1969.
2. Hoff, H. E. History of Physiology and Medicine. Lecture Series, Baylor College of Medicine, Houston, Texas, 1969.
3. Ross, J. N., Jr., Wieting, D. W., Hall, C. W., Kennedy, J. H., Garner, H. E., and DeBakey, M. E. Use of a Paracorporeal Left Ventricular Bypass Pump in Experimental Heart Failure. Amer. J. Cardiol. 27:12–19, 1971.
4. Ross, J. N., Jr., Alfrey, C. P., Lynch, E., and Kennedy, J. H. Hematologic Observations During Prolonged Partial Left Ventricular Bypass. Trans. Amer. Soc. Artif. Int. Organs, 17:195–199, 1971.
5. Ross, J. N., and Hill, W. A. Pre- and Postoperative Care of Calves Used in Cardiovascular Surgery. Presented at the 19th Annual Meeting of the American Association for Laboratory Animal Science, October 24, 1968, Las Vegas, Nevada.
6. Short, C. E., Keats, A. S., Liotta, D., and Hall, C. W. Anesthesia for Cardiac Surgery in Calves. Amer. J. Vet. Res. 29:2287–2294, 1968.
7. Donawick, W. J., Hiremath, I., Baue, A. E. Anesthesia, Ventilation and Experimental Thoracotomy in the Calf. Amer. J. Vet. Res. 30:533–541, 1969.
8. Nose, Y., Topaz, S., SenGupta, A., Tretbar, L. L., Kolff, W. J. Artificial Hearts Inside the Pericardial Sac in Calves. Trans. Amer. Soc. Artif. Int. Organs, 11:255–262, 1962.
9. Boncheck, L. I., Tatooles, C. J., Braunwald, N. S. Experimental Cardiac Surgery in the Calf. Ann. Thor. Surg. 3:211–217, 1967.
10. Reschly, L. J. and Dale, H. E. Effects of Ruminal Insufflation on the Circulatory System of Anesthetized Goats. Amer. J. Vet. Res. 31:279–283, 1970.
11. Van Zyl, J. J., Murphy, G. P., and de Klerk, J. N. Baboons in Transplantation Research at the University of Stellenbosch. Based upon a paper presented before the Association of Surgeons of South Africa, Cape Town, May, 1963.
12. Abildgaard, C. F., Harrison, J., and Johnson, C. A. Comparative Study of Blood Coagulation in Non-human Primates. J. Appl. Physiol. 30:400–405, 1971.
13. Lees, M. H., Herr, R. H., Hill, J. D. Distribution of Systemic Blood Flow of the Rhesus Monkey During Cardiopulmonary Bypass. J. Thorac. and Cardiovasc. Surg. 61:570–586, 1971.
14. Fell, C. Alteration in Tolerance of Hemorrhage due to Surgery and Duration of Anesthesia. J. Appl. Physiol. 28:178–182, 1970.
15. Fell, C. Changes in the Distribution of Blood Flow in Irreversible Hemorrhagic Shock. Am. J. Physiol. 210:863–868, 1966.
16. Short, C. E. Considerations in Anesthetic Management for Animal Experimentation. Anes. and Anal. Current Research, 49:125–131, 1970.
17. Cook, T. I., et al. Intermittent Positive Pressure Breathing and Extra-thoracis Assisted Breathing in Hypovolemia. Arch. Surg. 102:586–588, 1971.
18. Short, C. E., Cloyd, G. D., Ward, J. W. An Evaluation of Doxapram Hydrochloride to Control Respiration in Dogs During and After Initial Anesthesia. Vet. Med./Small Anim. Clin. pp. 787–790, Aug., 1970.
19. Geha, A. S., Salaymeh, M. T., Davis, G. L., and Baue, A. E. Replacement of the Aortic Valve with Molded Autogenous Grafts Grown in Response to Implanted Silastic. J. Thorac. and Cardiovasc. Surg. 60:661–672, 1972.
20. Priola, D. V., Fellow, C., Moorehouse, J., and Sanchez, R. Mechanical Activity of Canine Mitral Valve in situ. Am. J. Physiol. 219:1647–1651, 1970.
21. Iben, A. B., Pupello, D. F., and Holmes, S. L. The Histologic and Hemodynamic Evaluation of viable Heterograft Valves. J. Thorac. and Cardiovasc. Surg. 61:556–560, 1971.

22. Mohri, H., Reichenbach, D. D., Barnes, R. W., and Merendino, K. A. A Biologic Study of the Homologous Aortic Valve in Dogs. J. Thorac. and Cardiovasc. Surg. 54:622, 1967.
23. Williams, G. R., Seetapun, A., Shadid, E., Loughridge, B. P., and Hawley, D. H. The Use of Structured Fibrocollagenous Tissue as Partial and Complete Replacement of Cardiac Valves, in *Prosthetic Heart Valves*. Ed. Lyman A. Brewer III, Chas. C. Thomas Publishers, Springfield, Ill., p. 832, 1969.
24. Geha, A. S., Malt, S. H., Nara, Y., and Baue, A. E. Effect of Cardiopulmonary Bypass on Cerebral Metabolism. J. Thorac. and Cardiovasc. Surg. 61:200–211, 1971.
25. Goruin, A., Streisand, R. L., Stuckey, J. H. Total Cardiopulmonary Bypass, Myocardial Contractility and the Administration of Protamine Sulfate. J. Thorac. and Cardiovasc. Surg. 61:160–166, 1971.
26. Jaques, L. B. A Study of the Toxicity of the Protamine, Salmine. Brit. J. Pharmacol. 4:135, 1949.
27. Wallace, H. W., Corburn, R. F., Habboushe, F., Blakemore, W. S., Shepard, C. E. Mechanically Induced Intravascular Extravascular Hemolysis in Dogs. Circ. Res. 26:347–360, 1970.
28. Holdefer, W. F., Dowling, E. A., Kirklin, J. W. Hemodynamic, Metabolic and Organ Function Studies in Animals Subjected to Prolonged Veno-Arterial Bypass with the Bramson Membrane Lung. J. of Thorac. and Cardiovasc. Surg. 61:217, 1971.
29. Huggins, R. A., Smith, E. L., Deavers, S. Some Effects of Autologous and Homologous Blood or Plasma Overtransfusion in the Dog. Am. J. Physiol. 209:673–679, 1965.
30. Williams, B. T., Worman, R. K., Jacobs, R. R., and Schenk, W. G. An in vivo Study of Blood Flow Patterns Across the Normal Mitral Valve. J. Thorac. and Cardiovasc. Surg. 59:824–829, 1970.
31. Rittenhouse, E. A., Ito, C. S., Mohri, H., and Merendino, K. A. Circulatory Dynamics During Surface Induced Deep Hypothermia and After Cardiac Arrest for 1 Hour. Thorac. and Cardiovasc. Surg. 61:359–369, 1971.
32. Hurley, E. J., Dong, E., Stofer, R. C., and Shumway, N. E. Isotopic Replacement of the Totally Excised Canine Heart. J. Surg. Res. 2:90–94, 1962.
33. Cass, M. H., and Brock, R. Heart Excision and Replacement. Guy's Hospital Reports, 108:285, 1969.
34. Harrison, L. H., Jr., Hinshaw, L. B., Coalson, J. J., and Greenfield, L. J. Effects of E. Coli Septic Block on Pulmonary Hemodynamics and Capillary Permeability. J. Thorac. and Cardiovasc. Surg. 61:795–803, 1971.
35. O'Brien, D. J., Chapman, W. H., Rudd, F. V., and McRoberts, J. W. Carotid Artery Loop Method of Blood Pressure Measurement in the Dog., J. Appl. Physiol. 30:161–163, 1971.
36. Folts, J. D. Preparation of a Chronic Indwelling Catheter Using a Commercial Infant Feeding Tube. J. Appl. Physiol. 30:417–419, 1971.
37. Bache, R. J., Alexander, J. A., Rembert, J. C., and Greenfield, J. C. Coronary Hemodynamic Responses to Paired Ventricular Stimulata in the Awake Dog. J. Appl. Physiol. 29:145–150, 1970.
38. Jacobs, R. R., Williams, B. T., and Schenk, W. G., Jr. Cardiac and Systemic Toxicity of Hyperbaric Oxygen. J. Thorac. and Cardiovasc. Surg. 59:555–567, 1970.
39. Piermattei, D. L., and Swan, H. Techniques for General Anesthesia in Miniature Pigs. J. Surg. Res. 10:587–592, 1970.
40. Bustad, L. K. Pigs in the Laboratory. Science, 214:94, 1966.
41. McClellan, R. O. Applications of Swine in Biomedical Research. Lab. Anim. Care, 18:120, 1968.
42. Sawyer, D. C., Lumb, W. V., Stone, H. L. Cardiovascular Effects of Halothane, Methoxyflurane, Pentobarbital, and Thiamylal. J. Appl. Physiol. 30:36–43, 1971.
43. Swan, H. and Meagher, D. M. Total Body Bypass in Miniature Pigs. J. Thorac. and Cardiovasc. Surg. 61:956–967, 1971.
44. Swan, H., and Piermattei, D. L. Technical Aspects of Cardiac Transplantation in the Pig. J. Thorac. Cardiovasc. Surg. 61:710–723, 1971.
45. Piermattei, D. L. Autotransplantation of the Heart in Miniature Swine. A thesis presented in partial fulfillment of the requirements for the degree of Ph.D., Colorado State University, December, 1969.
46. Meagher, D. M., and Swan, H. Platelet Aggregation During the Collection and Storage of Porcine Blood. In Press.
47. Bulpitt, C. J., Dollery, C. T., Pallett, J. E., et al. Drug Infusion and Monitoring of Blood Pressure by Radio Telemetry in Unrestrained Pigs. Cardiovasc. Res. 4:520–528, 1970.
48. Weirich, W. E., Will, J. A., and Crumpton, C. W. A Technique for Placing Chronic Indweling Catheters in Swine. J. Appl. Physiol. 29:117–119, 1970.
49. Short, C. E. and Cloyd, G. D. The Use of Doxapram Hydrochloride with Inhalation Anesthetic in Horses—Part II. Vet. Med/Small Anim. Clin. 260–261, March, 1970.
50. Short, C. E., and Cloyd, G. D. The Use of Doxapram Hydrochloride Inhalation Anesthetic in Horses—Part I. Vet. Med./Small Anim. Clin. 157–160, February, 1970.
51. Lees, P., and Tavernor, W. D. The Influence of Suxamethonium on Cardiovascular and Respiratory Function in the Anesthetized Horse. J. Brit. Pharmacol. 36:116–131, 1969.
52. Tevik, A., Sharpe, J., Nelson, A. W., et al. Effect of Nitrogen in a Closed-Circle System with Low Oxygen Flows for Equine Anesthesia. J.A.V.M.A. 154:166–170, 1969.

53. Gillespie, J. R., Tyler, W. S., Hall, L. W. Cardiopulmonary Dysfunction in Anesthetized Laterally Recumbent Horses. Am. J. Vet. Res. 30:61–72, 1972.
54. Garner, H. E., Rosborough, J. P., Amend, J. F., and Hoff, H. E. The Grade Pony as a New Laboratory Model in Cardiopulmonary Physiology. Cardiovasc. Res. Cen. Bull. 9:91–104, 1971.
55. Garner, H. E., Rosborough, J. P., Amend, J. F. Normal Values for Blood Chemistry in the Pony. Southwest. Vet. 207–210, Spring, 1971.
56. Garner, H. E., Rosborough, J. P., Amend, J. F. and Hoff, H. E. Normal Ventilation and Acid-Base Values in the Laboratory Pony. Cardiovasc. Res. Cen. Bull. 9:160–166, 1971.
57. Garner, H. E., Rosborough, J. P., Amend, J. F. Laboratory Housing for the Pony in an Urban Research Facility. Am. Assoc. Lab. Anim. Sci. 21:269–271, 1971.
58. Rosborough, J. P., Garner, H. E., Amend, J. F. A Laboratory Restraining Device for the Pony. Am. J. Vet. Res. 32:1097–1098, 1971.
59. Dhindsa, D. S., Hoversland, A. S. and Kluempke, R. Halothane Semiclosed-Circuit Anesthesia in Pygmy and Large Goats. Am. J. Vet. Res. 31:1899, 1970.
60. Reschley, L. J. and Dale, H. E.: Effects of Ruminal Insufflation on the Circulatory System of Anesthetized Goats. Am. J. Vet. Res., 31:279–283, 1970.
61. Buck, W. B., Bond, K., and Lyon, N. C. A Technique for Permanent Cannulation of the Carotid Artery of Sheep and Goats. Cornell Vet. 55:154–163, 1965.
62. Stanley, T. H., and Boam, W. Use of the Computer to Monitor Long-term Cardiac Assistance. J. Thorac. and Cardiovasc. Surg. 57:519–526, 1968.
63. Cysewski, S. J. and Pier, A. C. Mycotic Abortion in Ewes Produced by *Asperigillus fumigatus:* Pathologic Changes. Am. J. Vet. Res. 29:1135–1151, 1968.
64. Akutsu, T., Takagi, H., Takano, H. Total Artificial Hearts with Built-in Valves. Trans. Amer. Soc. Artif. Int. Organs, 16:392–397, 1970.
65. Snow, H. D. and Tyner, J. G. Chronic Arterial and Venous Catheterization in Sheep. J. Vet. Res. 30:2241–2243, 1969.
66. Harrison, F. A. The Introduction and Maintenance of Permanently Indwelling Catheters in the Portal and Hepatic Veins of the Sheep. J. Physiol. London, 200:28–30, 1968.
67. Bensadoun, A. and Reid, J. T. Estimation of Rate of Portal Blood Flow in Ruminants: Effects of Feeding, Fasting, and Anesthesia. J. Dairy Sci. 45:540–543, 1962.
68. Annison, E. F., Hill, R. J., and Lewis, D. Portal Blood Analysis in the Study of the Absorption of the Ruminal Fermentation Products. Biochem. J. 60:19, 1955.
69. Wade, L., Jr. and Sasser, L. B. Body Water, Plasma Volume and Erythrocyte Volume in Sheep. Am. J. Vet. Res. 31:1375–1378, 1970.
70. Hansard, S. L., Butler, W. O., Comar, L. L. and Hobbs, C. S. J. Anim. Sci. 12:402–413, 1953.
71. DeVries, W. C., Kwan-Gett, C. S. and Kolff, W. J. Consumptive Coagulopathy, Shock, and the Artificial Heart. Trans. Amer. Soc. Artif. Int. Organs, 16:29–36, 1970.
72. McGilliard, A. D. An Implantable Hydraulic Needle for Sampling Blood or Infusion of the Portal Vein in Calves. J. Appl. Physiol. 30:297–301, 1971.
73. Good, A. L. and Stowe, C. M. Physiological and Biochemical Changes in Calves Induced by Experimental Bloat—Annual Progress Report to North Central Regional Committee for the Study of Bloat. Minnesota Agric. Exper. Sta. 1964.
74. Lanier, W. Lungworm Infection in Cattle. Vet. Med./Small Anim. Clin. 801–804, August, 1970.
75. Gourlay, R. N. and Leach, R. H. A New Mycoplasma Species Isolated from Pneumonic Lungs of Calves (Mycoplasma Dispar SP. NOV.) J. Med. Microbiol. 3:111–123, 1970.
76. Reisinger, R. C., Heddleston, K. L., Manthei, C. A. A Myxovirus (SF–4) Associated with Shipping Fever of Cattle. J.A.V.M.A. 135:147–152, 1959.
77. Moeller, Darby, J. What's New with Bovine Respiratory Problems. J.A.V.M.A. 154:1197–1200, 1969.
78. Tasker, J. B. Fluid, Electrolyte, and Acid-Base Abnormalities in Cattle. J.A.V.M.A. 155: 1906–1909, 1969.
79. Richard, J. L., Cysewski, S. J., Pier, A. C. Mycoflora of Bovine Lung, Placenta: Fetal Stomach. Am. J. Vet. Res. 31:995–998, 1971.
80. Baruah, H. J. The Air Spora of Cow Shed. J. Gen. Microbiol. 25:483–491, 1961.
81. Oehme, F. W. and Noordsy, J. L. Examination of Peritoneal Fluid in Differential Diagnosis of Bovine Diseases. Vet. Med./Small Anim. Clin. 54–59, January, 1970.
82. Campbell, G. S., Jones, E. W., Tavernor, W. D., et al.: Aorta to Pulmonary Artery Shunts. Arch. Surg. 99:723–727, 1969.
83. Alexander, A. F. and Jensen, R. Normal Structure of Bovine Pulmonary Vasculature. Am. J. Vet. Res. 24:1083–1093, 1963.
84. Detmer, D. E., Johnson, E. H., and Braunwald, N. S. Left Ventricular Apex-to-thoracic Aorta Shunts Using Aortic Valve Allografts in Calves. Ann. Thorac. Surg. 11:417–422, 1971.
85. Donawick, W. J., Nara, Y., Bave, A. E. Techniques for Succesful Cardiopulmonary Bypass in the Calf. J. Surg. Res. 9:79–85, 1969.
86. Gates, J. B., Botta, J. A., Teer, P. A. Blood Gas and pH Determinations in Cattle Anesthetized with Halothane. J.A.V.M.A. 158:1678–1682, 1970.

87. Detmer, D. E. and Braunwald, N. S. The Metal Poppet and the Rigid Prosthetic Valve. J. Thorac. and Cardiovasc. Surg. 61:175–180, 1971.
88. Gilbert, D. B., Nolan, S. P., Stewart, S., Fogarty, T., and Harris, E. K. An in vivo Study of Energy Losses of Ball and Disk Valves in the Mitral Position. J. Appl. Physiol. 28:282–290, 1970.
89. Chaplin, R. K., Waddern, D. E., Frost, O. L. Specific Gravity of Bovine Blood as Affected by Breed and Age. Am. J. Vet. Res. 31:1887–1888, 1970.
90. Sarin, C. L. Haemodynamic Studies in Calves Sustained on Artificial Hearts. Ann. Royal Coll. of Surg., England, 45:23–40, 1969.
91. Whitton, G. C. and Robertshaw, D. The Use of an Exteriorized Carotid Artery for Cardiovascular Studies in the Ox *(Box taurus)*. Am. J. Vet. Res. 26:183–185, 1965.
92. Betts, A. O., Jennings, A. R., Omar, A. R., Page, Z. E., Spence, J. B., Walker, R. G. Pneumonia in Calves Caused by Parainfluenza Virus Type 3. Vet. Rec. 76:382–384, 1964.
93. Bernhard, W. F., LaFarger, C. G., Hussain, M., Yamamura, N. and Robinson, T. C. Physiologic Observations During Partial and Total Left Heart Bypass. J. Thorac. and Cardiovasc. Surg. 60:807–817, 1970.
94. Shea, M. A. and Bernstein, E. F. Evaluation of the Functional Capability of the Baylor Left Ventricular Bypass Device. Trans. Amer. Soc. Artif. Int. Organs, 15:378–385, 1969.
95. Meyers, M. B., Cherry, G. Ventricular Area Thresholds in the Dog and Pig. J. Thorac. and Cardiovasc. Surg. 59:410–412, 1970.
96. Lumb, G. and Singletary, H. Blood Supply to Cardiac Conduction Tissue in Pig and Man. Surg. Forum, 12:218, 1961.
97. McKibben, J. S., Getty, R. A Comparative Morpholotic Study of the Cardiac Innervation in Domestic Animals.: Part I, Canine. Am. J. Anat. 122:533–544, 1968.
98. McKibben, J. S. and Getty, R. A Comparative Morphologic Study of the Cardiac Innervation in Domestic Animals. Am. J. Anat. 122:545–553, 1968.
99. McKibben, J. S. and Getty, R. Innervation of Heart of Domesticated Animals: Horse. Am. J. Vet. Res. 30:193–202, 1969.
100. McKibben, J. S., and Getty, R. Innervation of Heart of Domesticated Animals: Pig. Am. J. Vet. Res. 30:779–789, 1969.
101. McKibben, J. S. and Getty, R. A Comparative Morphologic Study of the Cardiac Innervation in Domestic Animals: Sheep. Anat. Anz. 1970.
102. Sisson, S. and Grossman, J. D. The Anatomy of Domestic Animals. W. B. Saunders Company, 1956, Phildelphia, London.
103. Kirkland, J. W. Pulmonary Diffunction After Open Heart Surgery. Med. Clin. N. Amer. 48: 1063, 1964.
104. Ghadiali, M. B., Horton, E. H. Experimental Extracorporeal Circulation in the Sheep. J. Thorac. and Cardiovasc. Surg. 59:710–716, 1970.
105. Geddes, L. A., Hoff, H. E. and McCrady, J. D. The Contribution of the Horse to Knowledge of the Heart and Circulation I: Stephen Hales and the Measurement of Blood Pressure. Conn. Med. 28: 795–800, 1965.
106. Geddes, L. A., Hoff, H. E., McCrady, J. D. The Contribution of the Horse to Knowledge of the Heart and Circulation II: Cardiac Catheterization and Ventricular Dynamics. Conn. Med. 29:864–874, 1965.
107. McCrady, J. D., Hoff, H. E. and Geddes, L. A. The Contribution of the Horse to the Knowledge of the Heart and Circulation IV: James Hope and the Heart Sounds. Conn. Med. 30:126–131, 1965.
108. Hoff, H. E., Geddes, L. A., McCrady, J. D. The Contribution of the Horse to Knowledge of the Heart and Circulation III: James Mackenzie, Thomas Lewis and the Nature of Atrial Fibrillation. Conn. Med. 30:43–48, 1965.
109. Bernhard, W. F., LaFarge, C. G., Jockin, H., Kitrilakis, S., Robinson, T. C. Chronic Left Ventricular Bypass with an Implantable Ventricular-Aortic Assist Pump. Circ. 50., 1:99–103, 1969.
110. LaFarge, C. G., Bernard, W. F. and Robinson, T. C. A Physiological Evaluation of the Baylor Left Ventricular Bypass. Proceedings of the Art. Heart Program Conference, Washington, D. C., 619–635, 1969.
111. Appleby, E. C., Graham-Jones, O., Keeble, S. A. Primate Diseases Infectious to Man. The Vet. Rec. (London), 75:81–85, 1963.
112. Asatiani, V. S. On Comparative Biochemistry of Man and Monkey. Uspekhi Sovremennoy Biologii, 44:313–333, 1957.
113. The Baboon, A New Star In Scientific Research. J. A. M. A. 186:22–34, 1963.
114. Editorial. Baboonery. New England J. Med. 269–983, 1963.
115. Belous, A. A., Magakyan, G. O. Experimental Pharmacotherapy of Hypertension and Coronary Insufficiency in Monkeys with Hexonium. Farmskologiya I Toksikologiya, 20:15–21, 1957.
116. Cheethan, R. W. S. Premature Artificial Systoles in the Baboon. South African Med. J. 8:739–740, 1934.
117. Florey, H. W., Greer, S. J., Poole, C. F., Werthessen, N. T. The Pseudointima Lining Fabric Grafts of the Aorta. Brit. J. Exp. Path. 42:236–246, 1961.
118. Franklin, D. L., Van Citters, R. L. Stroke Volume Changes in Baboons During Spontaneous Activity and Exercise. Am. J. Cardiol. 12:816–321, 1963.

119. Franklin, D. L., Watson, N. W., Van Citters, R. L. Smith, O. A. Blood Flow Telemetered From Dogs and Baboons. (Abstract) Federation Proceedings, 23:1214, 1964.
120. The Baboon—An Annotated Bibliography 1607–1964. Published by The Southwest Foundation for Research and Education, San Antonio, Texas, 1964.
121. Gilbert, C. and Gillman, J. Structural Modifications in the Coronary Artery of the Baboon (Papio Ursinus) with Special Reference to Age and Endocrine Status. S. African Journal of Med. Sci. 25:59–70, 1960.
122. Franklin, D. L., Watson, N. W., Van Citters, R. L. Blood Velocity Telemetered from Untethered Animals. Nature, 203:528–530, 1964.
123. Groover, M. E., Seljeskog, E. L., Haglin, J. J., Hitchcock, C. R. Myocardial Infarction in the Kenya Baboon Without Demonstratable Atherosclerosis. Angiology, 14:409–416, 1963.
124. The Baboon: An Annotated Bibliography, Supp. 1, 1964 and 1965. Published by the Southwest Foundation for Research and Education, San Antonio, Texas, 1965.
125. Katzberg, A. A. The Distribution of Elastic Elements in the Aorta and its Branchs in the Baboon, Papio Doguera. Anat. Rec. 151:489, 1965.
126. Cooke, J. P., Fife, W. P. and Bancroft, R. W. Comparative Cardiovascular Responses of Baboons & Dogs to Near-Vacuum Pressures. Brooks Air Force Base, Texas, U. S. Air Force School of Aerospace Medicine, 1968, iii + 7p (SAM–TR–68–44).
127. The Baboon—An Annotated Bibliography, Suppl. III, December, 1969. Published by the Southwest Foundation for Research and Education, San Antonio, Texas, 1969.
128. Davis, J. H., McPherson, B. R. and Moor-Jankowsi, J. Laboratory for Experimental Medicine and Surgery: Design and Operation. Laboratory Primate Newsletter, 7:5–12, 1968.
129. Field, W. E., Yelnosky, Y., Mundy, J., and Mitchell, Jr. Use of Droperidol and Fentanyl for Analgesia and Sedation in Primates. J.A.V.M.A. 149:896–901, 1966.
130. Foster, P. A., Groenewald, J. H., Schoones, R., VanZyl, J. J. W., Van Zyl, J. A. and Murphy, G. P. Anaesthesia for Baboons During Major Surgical Procedures—Observations During Renal Allotransplantation. S. Afric. Med. J. 42:1–3, 1968.
131. Willman, V. L., Hanlon, C. R., Kaiser, G. C., Cooper, T. Chronic Total Extrinsic Denervation of the Heart in Primates. Physiologist, 7:288, 1964.

DISCUSSION

Chairman Michael P. Kaye: Thank you Dr. Ross. This paper is now open for discussion.

Gerald E. Bisgard, University of Wisconsin: Have you encountered problems with metabolic acidosis in calves during or shortly after weaning? Also, what is your weaning regimen as concerns age, speed, and diet? You mentioned alfalfa pellets. What else are they on?

Dr. Ross: We wean the calves as soon as we get them. Most of our calves come directly off the range. When they're delivered at our preoperative facility, they're immediately placed on a ration containing alfalfa pellets and grain. So they're weaned acutely. We have not measured acid base balance in these animals.

Dr. Bisgard: About how old are they?

Dr. Ross: Three to four months.

Jeff Peters, Salt Lake City: We took the suggestion of Garner, Amend, Rosborough and Hoff of Houston and decided to use the horse for implantation of the total artificial heart. We've done four of them, and in our experience the horse is an extremely strong animal after implantation and still presents problems in handling.

Joel Cass, Veteran's Administration, Washington, D.C.: As the television ad tells us, we've come a long way. You couldn't have given this kind of information at a dignified research program and been looked upon as a scientist of note just a very few years ago. Such information was left in the laboratory books and never referred to as part of a research program, although funds and a good bit of the time of our investigators have been spent in obtaining it. When we now publish this information, we show the design of the facility and the techniques that we actually used. However, we seldom make mention of the modifications we have made during the course of our actual work. So the next fellow reading the original design says "We better do it that way because he's had some wonderful results." It would be helpful if our published papers included the modifications, or at least alluded to, the problems that the facilities and techniques have presented.

Chairman: Do you have any remarks in closing?

Dr. Ross: I think that Dr. Cass' remarks are well taken; it is true that we have not had open discussions of the tricks of the trade.

LEFT VENTRICULAR HEMODYNAMIC AND DIMENSIONAL RESPONSES TO TREADMILL EXERCISES IN NORMAL AND CARDIAC DENERVATED DOGS

E. B. Stinson, G. Rahmoeller, P. L. Tecklenberg, S. B. Colvin, K. W. Jones and J. E. Pierce*

Five normal (N) and eight chronically cardiac-denervated (D) (regional neural ablation) treadmill-trained dogs were studied during standardized five minute periods of treadmill exercise. Measurements included left ventricular pressure (minature solid-state pressure transducer), internal transverse LV diameter and derived velocity (piezoelectric crystals), and aortic flow (electromagnetic). Studies were performed in fully recovered animals one to three weeks after instrument implantation.

At rest there were no significant differences between N and D dogs in heart rate, LV systolic pressure, LV dp/dt, cardiac output, stroke volume, or peak LV ejection rate; average LV end-diastolic diameter was slightly greater in D than in N dogs (28.0 vs. 25.8 mm); the average maximum systolic rates of change of internal diameter (dD/dt) were similar in D and N dogs (78.8 vs. 79.5 mm/sec) and calculated peak circumferential velocities were comparable (3.4 vs. 3.9 circs/sec). With exercise N dogs showed near-maximal responses in all parameters within the first 30 seconds; LV end-diastolic diameter and pressure showed variable slight changes. Maximal responses in D dogs were attained gradually over the first three minutes of exercise, and lower maximal values were achieved for heart rate (D 193 vs. 250 beats/min), dD/dt (D 96 vs. N 110 mm/sec), peak V_{CF} (D 4.3 vs. N 5.7 circs/sec), cardiac output (D 5.1 vs. N 5.9 L/min), and LV dP/dt (D 5,100 vs. N 7,000 mmHg/sec). LV end-diastolic diameter and pressure consistently increased in D dogs. Increases in stroke volume occurred in both groups, but were proportionately higher in D dogs. These studies emphasize the importance of neural control for rate-dependent responses of the heart to muscular exertion.

INTRODUCTION

Experience with clinical heart transplantation has stimulated new interest in the function and adaptive mechanisms of the denervated heart. Donald and Shepard, on the basis of an extensive series of investigations of cardiovascular performance in cardiac-denervated dogs,[1,2] suggested that human cardiac recipients would suffer no physical limitations attributable to cardiac denervation *per se*.[2] Their experiments demonstrated an unchanged capacity for exercise after regional cardiac denervation, with a linear and quantitatively normal relationship between oxygen consumption and cardiac output during stress. They did note, however, striking differences between normal and denervated dogs in the responses of heart rate and stroke volume at all levels of exercise. Whereas normal animals elevated cardiac output almost entirely by means of prompt increases in heart rate, denervated dogs utilized principally increases in stroke volume, especially during mild exercise.[2]

Clinical experience has substantiated the expectation that heart recipients can sustain ordinary physical activity without undue cardiac symptoms.[3] Cardiac catheterization studies in eight patients one and two years after transplantation, however, have shown that although the output of the transplanted heart rises linearly with increasing oxygen consumption during supine submaximal exercise, the absolute level of cardiac output is in the low normal range or subnormal, both at rest and during exertion.[4] Increases in both heart rate and stroke volume contributed to the augmentation of cardiac output although the effect of stroke volume was predominant. Left ventricular end-diastolic pressure, which was normal at rest in all patients, promptly increased to abnormal levels with the onset of exercise, and remained elevated despite a gradual rise in heart rate to levels comparable to normal. Some patients exhibited a significant increase in stroke volume and mean left ventricular ejection rate, in association with slightly diminished left ventricular end-diastolic pressure, after several minutes of steady exercise, suggesting a gradual enhancement of contractile state.

We have attempted to further define differ-

* Clinic of Surgery, National Heart & Lung Institute, Bethesda, Maryland 20014

ences between the normal and denervated left ventricular response to exercise in dogs by simultaneous measurements of ventricular pressure, internal transverse diameter, and aortic flow.

METHODS

Seventeen American foxhounds, weighing 15 to 23 Kg, were trained to run freely on a motor-driven treadmill. After successful training, a left thoractomy was performed under sterile conditions. A miniature solid-state pressure transducer (7 mm diameter) was inserted into the left ventricular cavity through a small incision at the apex, and fixed to the epicardium by a pursestring suture. The gauge was positioned approximately 8 millimeters inside the endocardial surface to avoid muscular entrapment during systole. A snug-fitting electromagnetic flow transducer was positioned around the ascending aorta over a thin layer of Dacron mesh which effectively prevented aortic rupture due to erosion, but did not significantly affect calibration.

For measurement of internal left ventricular diameter two 5 millimeter discs of lead titanate-zirconate piezoelectric crystal, mounted in lucite housings and capped with convex lucite lenses, were positioned on opposite endocardial surfaces of the left ventricle across the maximum transverse diameter in an anteroposterior orientation. Proper placement of the transducers was determined by palpation with the operator's index finger inserted through the left atrial appendage and mitral valve. After palpation, a 14 inch length of PE 240 tubing, through whose terminal portion a 6 inch, 17 gauge needle had been inserted, was passed through the ventricle across the selected diameter. Following transfixion of the ventricle, the needle was withdrawn and a loop of PE tubing was pulled through the mitral valve and exteriorized via the left atrial appendage. The loop was then cut and the terminal portions of the transducer leads were inserted into the open ends of the PE tubing. By withdrawing the other ends of the PE tubing from the ventricular surfaces the transducers were pulled into the ventricle and positioned against the endocardium. Proper placement and avoidance of chordal structures were again assured by palpation within the ventricle. The left atrial appendage was then ligated. Inflow occlusion was not employed, and blood loss was negligible.

At the conclusion of operation, all lead wires were passed subcutaneously from the chest incision to the back of the neck where they were exteriorized and bandaged. In 11 of the 17 dogs regional cardiac denervation was performed immediately prior to the implantation of instruments. There was no significant difference in body weights between normal and denervated dogs (20.5 vs. 21.7 Kg). Denervation was accomplished essentially as described by Cooper[5] except that the caudal third of pericardium was not removed and the inferior pulmonary veins were not denuded. Criteria for verification of denervation included absence of sinus arrhythmia, lack of a heart rate startle response, absence of heart rate changes with atropinization (2 mg) or administration of tyramine (.06 μg/Kg), and in three animals, absence of heart rate or contractile responses to supramaximal stimulation of the cervical vagi and stellate ganglia under pentobarbital anesthesia.

All animals were treated with penicillin and streptomycin post-operatively, and were subsequently studied 10 days to four weeks after operation, following clinical recovery. Daily rectal temperatures were also recorded early post-operatively, and, in the presence of fever, chloromycetin was also given for 5–7 days.

Following recovery, animals were exercised on a treadmill at least three times at daily intervals before study. At the time of study, a standard five minute run on the treadmill at 3.5 mph and 15° grade was performed. All animals easily completed this workload which was chosen to represent light exercise.[6-8] Control measurements were obtained with dogs standing on the treadmill immediately before the start of the run. Interval records during the run were obtained at 30 seconds, one minute, and at one minute periods thereafter for the duration of the run. Additional recordings were made at one and five minutes during recovery. Signals were recorded on a Brush 480 recorder at paper speeds of 25 and 100, and in some cases 200 mm./sec., and also on magnetic tape (Ampex

FR 1300). Animals which balked during the study or pulled against the leash were excluded from analysis.

The pressure transducers were calibrated before and after implantation *in vitro* at 38° C. Static calibration against a mercury column was found to be constant and linear from zero to 300 mm Hg (± 1%) without hysteresis, and the frequency response of the pressure recording system was linear to 80 cps (± 5%). Zero stability, though constant with some gauges, was not found to be reliable; thus, changes in left ventricular end-diastolic pressure (LVEDP) are reported as deviations from a preset baseline arbitrarily set to 4-5 mm Hg (catheterization studies in several dogs showed LVEDP to range from 2 to 8 mm Hg). Since zero baselines *in vitro* were found to be stable over a minimum period of two hours in the laboratory, delta values for LVEDP during the 10 minute periods of study were considered to be valid. The rate of change of left ventricular pressure (LV dp/dt) was obtained with an active differentiator having a linear frequency response and constant phase error of two msec to 80 cps. Pressure and other measurements were averaged over several cardiac cycles selected during expiratory pauses.

Aortic flow was measured with a Biotronex Model 610 Pulsed-Logic flowmeter whose static calibration was linear to 20 L/min and whose frequency response was linear to 50 cps (± 8%). Flow transducers were calibrated pre- and postimplantation, and in some instances *in situ* after sacrifice of the animal. Late diastolic flow was taken to represent zero flow, and stroke volume was obtained by electronic integration.

For the determination of left ventricular diameter the transmitting crystal was pulsed for one microsecond at 5.6 KHz. The received signal from the opposite crystal was rectified, amplified, and detected by a tracking gate similar to that employed in radar tracking systems.[9] The analog output of the tracker was proportional to distance between the crystals (transmission time) and was precalibrated *in vitro*. After low pass filtering the diameter signal was differentiated with a passive differentiator to obtain instantaneous rate of change of diameter. Maximum delay through both tracker and filter was approximately 10 msec and was constant up to 20 cps. End-diastolic diameter (D_d) was measured at the beginning of isovolumic systole, and end-systolic diameter (D_s) was taken as the minimum diameter achieved during systole. Percent systolic shortening of internal diameter was calculated as $D_d - D_s/D_d$. The rate of diameter change (dD/dt) was measured directly from the differentiated diameter signal; maximum systolic − dD/dt, which occurred at or shortly following peak aortic flow, was divided by the instantaneous diameter to obtain peak circumferential fiber shortening rate, V_{CF}, in circs/sec $(-\pi dD/dt)\ /\pi D$.

Within- and between-groups means were compared by the appropriate Student's T-test, assigning a two-tailed significance level to the P value.

At necropsy there was no evidence of intrathoracic infection in animals included in the study. Adhesions between the lungs and heart were routinely present, and the intrathoracic portions of the cables were encased in fibrous tissue. The intraventricular transducers were covered with small fibrinous thrombi in the majority of animals, but the presence of such thrombi did not correlate with transducer malfunction.

RESULTS

Control State

Under control conditions, with dogs standing on the treadmill, there were no significant differences between normal and denervated animals in stroke volume, cardiac output, developed LV pressure, peak LV dp/dt, maximum rate of systolic diameter shortening ($-dD/dt$), or V_{CF} (Table I). Mild tachycardia was present in normal animals (average 128 beats/min.) as well as in denervated subjects (average 140 beats/min. $P > .05$). Average end-diastolic diameter was greater in the denervated animals (28 vs. 25.8 mm), but this difference was not statistically significant. There was, however, a significant difference between denervated and normal dogs in percent shortening of transverse diameter during systole, with normal animals

TABLE I.—*Left Ventricular Hemodynamic and Diameter Changes Treadmill Exercise at 3.5 MPH and 15° for Five Minutes*

		Control	30 Secs.	1 Min.	2 Min.	3 Min.	4 Min.	5 Min.	1 min. After	5 min. After
HR	N	128 (9.8)	225 (8.2)	238 (8.6)	243 (9.8)	243 (9)	250 (6.1)	248 (6.9)	161 (9)	141 (10.6)
	D	140 (5.1)	162 (6.3)	177 (6.3)	190 (7.2)	192 (6.6)	193 (6.6)	193 (6.6)	171 (5.4)	150 (4.2)
SV	N	22.1 (1.2)	23.9 (1.8)	24.9 (1.9)	24.8 (2)	25.2 (2)	24.7 (2)	24.6 (2.1)	21.7 (2.1)	22.2 (1.3)
	D	21.9 (1.1)	25.9 (1.7)	25.8 (1.6)	26.2 (1.3)	26.7 (1.3)	26.4 (1.3)	26.1 (1.4)	23.3 (.9)	21.9 (1.1)
CO	N	3.1 (.3)	5.3 (.5)	5.7 (.5)	5.8 (.5)	5.9 (.6)	5.9 (.6)	5.8 (.5)	3.6 (.5)	3.3 (.4)
	D	3.1 (.2)	4.2 (.3)	4.5 (.3)	4.9 (.3)	5.1 (.2)	5.0 (.2)	5.0 (.2)	3.9 (.1)	3.3 (.3)
D_d	N	25.8 (2.1)	25.3 (2)	25.6 (1.9)	25.6 (1.9)	25.8 (1.8)	25.7 (1.8)	25.8 (1.8)	26.7 (2.2)	26.6 (1.9)
	D	28.0 (1.6)	28.6 (1.7)	29.0 (1.5)	28.7 (1.6)	28.2 (1.6)	28.3 (1.5)	28.4 (1.5)	28.2 (1.6)	27.9 (1.6)
LVEDP	N	—	0.5 (1.6)	2.8 (1.4)	3.8 (1.8)	3.8 (1.9)	4.8 (2.2)	4.8 (2.2)	3.5 (1.7)	1.8 (1.2)
	D	—	5.2 (1.1)	5.8 (.8)	5.5 (1.6)	5.0 (1.5)	4.2 (1.6)	3.9 (1.6)	1.6 (.5)	0.6 (.8)
LVP	N	159 (9.4)	189 (8.2)	197 (.9)	200 (10.2)	202 (11.4)	200 (10.6)	195 (9.8)	177 (12.7)	162 (8.2)
	D	137 (5.4)	139 (7.2)	152 (7.8)	163 (7.5)	165 (7.5)	165 (8.1)	163 (8.4)	159 (7.2)	141 (6.3)
LV dp/dt	N	3280 (313)	6285 (609)	6980 (604)	7390 (680)	7410 (828)	7060 (790)	6800 (806)	4075 (708)	3557 (333)
	D	2865 (227)	3630 (412)	4220 (470)	5106 (666)	5104 (594)	5042 (703)	4700 (600)	3956 (490)	3000 (241)
Max −dD/dt	N	80 (5)	105 (5.1)	109 (5.2)	106 (6)	109 (8.7)	113 (9.6)	113 (8.2)	83 (7.7)	74 (4.6)
	D	79 (5.2	87 (5.9)	91 (5.4)	96 (4.7)	96 (6.6)	96 (5.4)	94 (6.3)	79 (3.6)	78 (4.5)
V_{CF}	N	3.9 (.5)	5.6 (.8)	5.6 (.7)	5.6 (.8)	5.5 (.8)	5.7 (.9)	5.7 (.8)	3.9 (.6)	3.5 (.5)
	D	3.4 (.3)	3.8 (.3)	4.0 (.2)	4.2 (.2)	4.3 (.4)	4.3 (.3)	4.3 (.4)	3.4 (.3)	3.4 (.3)
% Sys. Short.	N	34.5 (3.7)	39.8 (4.6)	38.8 (4.7)	39.5 (4.9)	39.3 (4.8)	38.5 (4.9)	38.8 (4.5)	32.3 (3.4)	34.8 (3.6)
	D	25.1 (1.5)	29.1 (1.6)	30.6 (2.1)	31.3 (2.2)	31.3 (2.3)	30.3 (1.8)	29.7 (1.8)	24.9 (1.4)	23.7 (1.4)

N = Normal; D = Denervated; Numbers in parentheses indicate ± S.E.M; HR = Heart rate (beats/min.); SV = Stroke volume (ml/beat); CO = Cardiac output (L/min); D_d = End-diastolic diameter (mm); LVEDP = Left ventricular end-diastolic pressure change from control (mm Hg); LVP = LV systolic pressure (mm Hg); LV dp/dt = LV rate of pressure change (mm Hg/sec); Max −dD/dt = Maximum rate of diameter shortening during systole (mm/sec); V_{CF} = max −dD/dt divided by instaneous diameter (cirs/sec); % sys. short. = Percent decrease in diameter during systole.

showing a relatively greater stroke change in diameter.

Responses During the First Minute of Exercise

With the onset of exercise normal animals exhibited abrupt increases in heart rate, cardiac output, peak LV dp/dt, −dD/dt, and V_{CF} which reached nearly maximal levels with 30 seconds to one minute ($P < .05$ for all) (Table I). Average end-diastolic diameter decreased initially ($P > .05$), and the mean change in LVEDP was +0.5 mm Hg ($P > .05$) at 30 seconds, followed by a slight rise. Stroke volume increased only slightly ($P > .05$). Percent systolic shortening of transverse diameter rose from 34.5 to 39.8% ($P < .02$).

In contrast, denervated dogs showed an initial increase in end-diastolic left ventricular diameter ($P < .05$), associated with a significantly higher increment in LVEDP (+5.2mm Hg) ($P < .05$). Stroke volume increased 19 percent ($P < .01$), from 21.9 to 25.9 ml/beat.

Heart rate, peak LV dp/dt, and percent systolic diameter shortening showed gradual but significant increases above control at 30 seconds and one minute ($P < .05$ for all). Increases in systolic − dD/dt and V_{CF} were of borderline significance at 30 seconds, but were significant at one minute. Developed LV pressure remained near control level at 30 seconds, and thereafter showed a gradual rise ($P > .05$) by one minute.

In summary, during the first 30 to 60 seconds of exercise, normal dogs showed significantly greater increments and absolute values for heart rate, cardiac output, developed LV pressure, LV dp/dt, and maximum systolic −dD/dt and V_{CF}. Increases in percent systolic diameter shortening were roughly comparable in the two groups, although normal animals exhibited an initial decrease in end-diastolic diameter, while denervated dogs showed the opposite. Augmentation of stroke volume was significantly greater in the denervated group.

Two to Five Minutes of Exercise

After the first minute of running, normal dogs showed no further significant changes in the parameters analyzed.

Denervated animals, in contrast, demonstrated additional and significant elevation of heart rate, cardiac output, developed LV pressure, LV dp/dt, and maximum systolic $-dD/dt$ and V_{CF}.

Maximal mean values in these parameters were attained between two and three minutes of exercise. At this time differences between denervated and normal dogs for cardiac output, maximum systolic $-dD/dt$ and V_{CF}, and percent systolic shortening of transverse diameter were statistically insignificant, although mean values tended to be higher for normal subjects (Table I). Furthermore, end-diastolic diameter and LVEDP showed slight decreases in denervated dogs during late exercise, but these trends were of borderline statistical significance.

Recovery

Following cessation of exercise, denervated dogs showed proportionately more gradual decreases in all parameters, and, in fact, reversal of differences between normal and denervated subjects was observed one minute after exercise for heart rate and cardiac output ($P > .05$). LV diameter tended to increase immediately after exercise in normal animals, whereas in denervated dogs it tended to decrease. By five minutes after exercise, return to or near control levels was observed for all parameters in both groups.

DISCUSSION/SUMMARY

The results of these studies confirm the ability of the regionally denervated heart to respond in a directionally appropriate manner to the metabolic demands of muscular exercise. They also illustrate the primary dependence of the denervated heart on the Frank-Starling mechanism as a means of increasing output, especially in the early stages of exertion. In general, however, the pumping performance (output) of denervated hearts during running was observed to be slightly inferior to that of normal hearts, even though comparable levels were attained. It is not clear why this discrepancy with the data of Donald and Shepard, who found no compromise of steady-state cardiac output in denervated dogs during light exercise,[1] was observed. Others, though, have noted substantial differences between denervated and normal animals in cardiac output during treadmill exercise.[10] Possible factors in the present study include the breed of animal used and degree of training, differences in oxygen consumption or actual work performed between normal and denervated dogs, and the influence of excitement during the control state.

Although oxygen consumption was not measured in the present studies, a consistent attempt was made to insure that all subjects ran freely on the treadmill without restraint or pulling at a constant speed and elevation. Such standardization of effort has been shown to result in comparable levels of oxygen consumption during work in different animals and from run to run.[6] The effects of excitement on cardiovascular performance are difficult to differentiate from physiological responses to exercise, but have been shown by Barger to be associated with higher cardiac outputs both at rest and during running in normal dogs.[6] The mild tachycardia and relatively high stroke change in diameter (percent systolic shortening) seen in our normal dogs immediately prior to running suggest that excitement may indeed have been present and may have influenced the absolute level of cardiac output achieved during exercise. Obviously, the effects of excitement would tend to be attenuated in dogs deprived of direct neural cardiac control.

In addition to the initial directional changes in left ventricular diameter and end-diastolic pressure at the onset of exercise, denervated dogs differed markedly from normal in the early acceleration of myocardial contraction velocity. Indeed, during the first 30 seconds of running minimal increases in the rate of diameter shortening occurred, and maximal improvement did not develop until the second or third minute of running. In contrast, normal animals showed rapid acceleration of this function, as well as other rate-dependent parameters, to maximal or near maximal levels within the first 30 seconds. The gradual augmentation of contraction velocity in denervated dogs was associated with parallel elevation of heart rate and cardiac output. Such observations suggest a

gradual enhancement of myocardial contractile state in the denervated animals throughout the first few minutes of running, due possibly to the operation of a humoral mechanism such as circulating catecholamines. This hypothesis is being further examined by analysis of instantaneous velocity-length-tension relationships and by observation of the effects of beta-adrenergic blockage prior to exercise.

REFERENCES

1. Donald, D. E., and Shephered, J. T. Responses to exercise in dogs with cardiac denervation. Am. J. Physiol. 205:393–400, 1963.
2. Donald, D. E., and Shephered, J. T. Sustained capacity for exercise in dogs after complete cardiac denervation. Am. J. Cardiol. 14:853, 1964.
3. Stinson, E. B., Griepp, R. B., Clark, D. A., Dong, E., Jr., and Shumway, N. E. Cardiac transplantation in man. VIII. Survival and function, J. Thorac. and Cardiovasc. Surg. 60:303, 1970.
4. Stinson, E. B., Griepp, R. B., Schroeder, J. S., Dong, E., Jr., and Shumway, N. E. Hemodynamic observations one and two years after cardiac transplantation in man. Circulation. In press.
5. Cooper, T., Gilbert, J. W., Bloodwell, R. D., and Crout, J. R. Chronic extrinsic cardiac denervation by regional neural ablation. Description of the operation, verification of the denervation, and its effects on myocardial catecholamines. Circulation Res. 9:275–281, 1961.
6. Barger, A. C., Richards, V., Metcalfe, J., and Gunter, B.: Regulation of the circulation during exercise. Cardiac output (Direct Fick) and metabolic adjustments in the normal dog. Am. J. Physiol. 184:613, 1956.
7. Bailie, M. D., Robinson, S., Rostorfer, H. H., and Newton, J. L. Effects of exercise on heart output of the dog. J. Appl. Physiol. 16:107, 1961.
8. Cerretelli, P., Piiper, J., Mangili, F., Cuttica, F., and Ricci, B. Circulation in exercising dogs. J. Appl. Physiol. 19:29, 1964.
9. Schuette, W. H., and Simon, A. L. A new device for recording cardiac motion. Med. Res. Eng. 7:25, 1968.
10. Ashkar, E., and Hamilton, W. F. Cardiovascular response to graded exercise in the sympathectomized vagotomized dog. Am. J. Physiol. 204:291, 1963.

DISCUSSION

Chairman Hamlin: Did you have an opportunity to measure diastolic compliance or systemic arterial pressures in any of these that might help account for the increase in end diastolic volume in the denervated dogs?

Dr. Stinson: In this particular group of animals we didn't make any attempt to analyze diastolic ventricular compliance. In a few animals, in the initial portion of the series, arterial pressures were measured concomitantly with the other parameters. Changes in arterial pressure with the onset of exercise were similar to peak developed left ventricular pressures that I illustrated. Interestingly, it showed very minimal, if any, increase with the onset of running. This sort of resembles the response of a normal animal who has been given propanolol prior to exercise.

Dr. Hamlin: Why do you suppose end diastolic volume and pressure increased?

Dr. Stinson: Well, there are several lines of evidence which show that the metabolic vasodilatation associated with the onset of exercise does results in an increase in ventricular filling or venous return, if you will. In the absence of elevation of ventricular afterloads, then I must assume that it's an increase in preload which accounts for the initial changes in diameter and pressure. In addition to the metabolic vasodilatation associated with the onset of muscular exercise, Guiden has shown that there is a translocation of blood from peripheral pools to the central circulation with the onset of muscular exertion. It's my assumption that this accounts for the increased ventricular filling.

D. C. Sawyer, Michigan State, East Lansing: Since you set out to do this study as a comparison to those human transplanted patients, why did you select the dog and did you consider species other than the dog?

Dr. Stinson: Well, we considered man of course but it's a little more difficult to measure these parameters in man. I've not had the experience to seriously entertain other species of animals for treadmill exercise. Do you have any suggestions?

Dr. Sawyer: The pig runs great on a treadmill.

Chairman: Did you try to simulate Donald's and Shepherd's studies by exercising dogs with denervation plus propanolol?

Dr. Stinson: Yes we did. Although these studies are in the initial stages of analysis at

this time. We found that in contrast to the findings of Donald and Shepherd a healthy dose of propanolol in a denervated dog very, very seriously impairs his response to running, whether one is considering mild or severe exertion. We also found almost complete aggregation of most of the rate dependent parameters during running.

SINGLE LUNG TRANSPLANTATION WITH IMMEDIATE LIGATION OF THE OPPOSITE PULMONARY ARTERY IN THE DOG: A MODEL FOR ASSESSING THE FUNCTIONAL ADEQUACY OF TRANSPLANTED LUNGS

F. J. Veith, S. B. P. Sinha,* S. S. Siegelman and J. W. C. Hagstrom

Since single lung transplants in patients will have to provide virtually total pulmonary function, it is important that studies on experimental lung transplants be performed under analogous functional stresses. We found that dogs routinely survive after autotransplantation or allotransplantation of either lung and immediate ligation of the opposite pulmonary artery if technical errors are avoided and if the arterial anastomosis of the transplant is made distensible. This can be achieved by a variety of techniques. Nine of 16 dogs (56%) subjected to autotransplantation of the left lung and immediate ligation of the right pulmonary artery (RPA) survived from 2 to 45 months. These dogs had pulmonary artery pressures, pulmonary vascular resistances, arterial and venous blood gas values, and lung histology comparable to those in normal dogs undergoing RPA ligation. The only abnormality present on pulmonary angiograms was a 25–75% increase in the diameter of the left pulmonary artery and its major branches. This documents the importance of distensibility of the large pulmonary arteries in situations requiring increased pulmonary blood flow and emphasizes the importance of making a distensible pulmonary artery anastomosis in dog lung transplants charged with carrying the entire cardiac output. Only 11 of 115 dogs (10%) receiving left lung allografts with immediate ligation of the RPA lived one to three months. Despite intensive immunosuppression with azathioprine, antilymphocyte serum, and corticosteriods, rejection was a major cause of death in these dogs. Thus, a single transplanted canine lung can provide total respiratory and vascular function at all times after operation up to 3 years. The described model supports the feasibility of single lung transplantation in patients with pulmonary hypertension and provides a method for evaluating immunosuppressive regimens and lung preservation techniques intended for use in lung transplantation.

INTRODUCTION**

Potential lung transplant candidates will have severe bilateral pulmonary disease. Accordingly, any therapeutic lung transplant must be able to provide adequate total gas exchange while carrying the entire cardiac output at tolerable pulmonary arterial pressures. Since there are presently no methods for providing assisted gas exchange, the transplanted lung must meet these functional requirements from the moment of its insertion onward. Because of the complexity of bilateral lung transplantation procedures, it would be advantageous if a single lung graft could satisfy these needs.

To be of maximal predictive value for human lung transplantation, studies of experimental lung transplants should be performed under functional stresses analogous to those which would confront the human single lung graft. Transplantation of one lung followed immediately by ligation of the pulmonary artery to the opposite lung in an experimental animal would force the transplant to provide total gas exchange while carrying the entire cardiac output.

Numerous investigators had attempted to do this without success in experimental animals prior to 1969, although reimplantation of one lung followed by delayed ablation of function in the opposite lung had been accomplished in dogs[1,2] and baboons.[3] The inability to achieve survival after single lung transplantation and immediate contralateral pulmonary artery ligation had been blamed on the fixed and elevated transplant vascular resistance which had been though to be a consequence of denervation. When the transplant with this fixed resistance was forced to accept the entire pulmonary blood flow, it was thought that intolerable right heart failure or fatal pulmonary insufficiency secondary to damage to the pulmonary microvasculature would result.

In 1969, we reported the following observa-

* Department of Surgery, Montefiore Hospital and Albert Einstein College of Medicine, 111 East 210 Street, New York 10467.

** This work was supported in part by grants from the U.S. Public Health Service (HE 11472), the Health Research Council of the City of New York, the Manning Foundation and the John A. Hartford Foundation.

tions: (1) that the usual pulmonary artery anastomosis can, under certain circumstances, contribute to the high vascular resistance which occurs in transplanted canine lungs; (2) that the fixed nature of the vascular resistance in dog lung transplants is largely due to the failure of the arterial anastomosis to dilate, as the normal pulmonary artery does in response to increased flow; (3) that lung transplants with wide distensible pulmonary artery anastomoses can have a normal decrease in vascular resistance with increasing flows; and (4) that dogs receiving a lung autograft or allograft with such a distensible anastomosis can tolerate simultaneous ligation of the contralateral pulmonary artery and can survive solely on the vascular and respiratory function of the transplanted lung.[4,5,6]

The present communication will attempt to answer some of the questions and objections that have been raised in connection with our model of single lung transplantation and immediate ligation of the opposite pulmonary artery in the dog. In addition, we will present new findings which elucidate the efficacy of presently available immunosuppression in preventing the functional defects of lung allograft rejection and which relate to the long-term fate, morphology, and functional capabilities of autografted lungs that are providing total gas exchange and carrying the entire cardiac output for periods up to 45 months.

METHODS

Left lung autografts were performed in 16 mongrel dogs, and left lung allografts were performed in 115 mongrel dogs. Surgical techniques for these operative procedures have been described elsewhere.[5,7] Key features of the operative procedures included: (1) a two-layered everting atrial anastomosis which excludes atrial muscle from the lumen and prevents thrombosis, (2) a telescoped bronchial anastomosis, and (3) a distensible pulmonary artery anastomosis. Within ten minutes of completion of the transplant the right pulmonary artery was doubly ligated thereby placing total functional dependency on the transplant.

All animals with allotransplants received immunosuppression. This included 1–3 mg/kg/day of azathioprine and varying doses of rabbit antidog lymphocyte serum (ALS) and corticosteroids (Table I).

All autografted and selected allografted recipient dogs underwent serial studies of their pulmonary hemodynamics, arterial and venous blood gases, and pulmonary angiographic morphology. These studies were carried out preoperatively in most animals and at two to six month intervals up to 45 months postoperatively. The methodology and calculations used in these studies have been described in detail elsewhere.[5,8] Briefly, the animals were anesthetized with pentobarbital, the femoral artery was cannulated, and a polyethylene catheter was introduced by way of the external jugular vein and fluoroscopically guided into the pulmonary artery. While the dogs were breathing room air spontaneously through an endotracheal tube, the pulmonary artery pressure and cardiac output were determined; pO_2, pCO_2, and pH of blood from the pulmonary and femoral arteries were measured; and oxygen saturations and left lung vascular resistance were calculated. Then, after the injection of 25 milliliters of 60 per cent diatrizoate meglumine (Renografin) into the main pulmonary artery, 12 serial roentgenograms were taken at half second intervals with the use of a mechanical cassette changer.

Complete autopsies were performed on all dogs that died. All anastomoses and the site of right pulmonary artery ligation were inspected

TABLE I.—*Immunosuppressive Regimens and Survival Results in Groups of Dogs Receiving Left Lung Allografts with Right Pulmonary Artery Ligation*

Group Number	No. of Dogs	Azathioprine (mg/kg/day)	ALS (ml/day)	Prednisone (mg/day)	Mean Survival (Days)	No. Dogs Surviving >1 month
1	33	1–2	3–9	—	16	6
2	21	1–2	10–19	—	6	2
3	61	2–3	20–50	50–75	6	3

grossly. The lungs were inflated with 10 per cent formalin instilled in the trachea at a pressure of 15 centimeters of water. They were then immersed in formalin, sectioned, and stained with hematoxylin and eosin.

RESULTS

Survival with Autografts

Of the 16 dogs that underwent left lung autotransplantation and right pulmonary artery ligation, four died during the first postoperative week, one from a vascular thrombosis, one from pneumonia, and two of unknown causes. Three dogs lived from one to four weeks, and two of these dogs died of infarction and pneumonia in the right lung. The cause of death in the third dog was unknown. Two of the nine dogs which survived more than two months died during subsequent studies, one at four months and one at 14 months. Two others died of pneumonia or infarction of the right lung, or both, three and six months after operation. One old dog died of unknown causes 19 months after operation, and another animal died of unknown causes 30 months after operation. The remaining three dogs have tolerated repeated studies and are alive and well with normal exercise tolerance and activity patterns, 33 to 45 months after operation. One pair of long-term survivors has produced two normal litters.

Survival with Allografts

Although the animals receiving lower dose immunosuppression (group 1) appeared to fare better, there was no significant difference in survival between the various groups of immunosuppressed animals receiving left lung allografts with right pulmonary artery ligation (Table I). Therefore, the fate of all 115 animals can best be described as a single group. Eleven (10%) of the animals lived more than one month; six (5%) lived more than two months; but only one survived over three months. Although 16 of the 104 deaths within one month of operation were the result of technical problems such as atrial thrombosis or bronchial dehiscence with leakage, many more (52) were due to a combination of pneumonia and allograft rejection involving the transplanted lung. Fifteen animals died with an essentially normal transplant but with severe pneumonia and/or infarction of their own right lung. Twenty-one of the deaths within one month of operation were not associated with any significant morphologic abnormality in either lung. Toxicity of the immunosuppressive therapy may have contributed to the death of these animals.

Six of the animals surviving more than one month died because of the effects of chronic allograft rejection on the transplant. One animal died from pneumonia and/or infarction of his right lung. However, four animals had no satisfactory morphologic cause of death either in their lungs or other organs. These latter four animals had lungs that were histologically normal in most areas except for slight mononuclear cell infiltration of their allograft (Figure 1).

Hemodynamic and Blood Gas Studies

Pulmonary artery mean pressure increased significantly with the approximate doubling of blood flow through the left lung brought about by ligation of the right pulmonary artery (Table II). However, this increase was not di-

TABLE II.—*Hemodynamic and Blood Gas Data After Autotransplantation of the left Lung and Right Pulmonary artery ligation*

	No. of dogs	Pulmonary artery pressure (mm Hg)	Left Lung Vascular resistance (dynes sec/cm^5)	Arterial $p0_2$ (mm Hg)	Arterial pCO_2 (mm Hg)	Arterial pH	Arterial Oxygen Saturation (per cent)	Venous Oxygen Saturation (per cent)
Preoperative	16	15 ± 1	865 ± 92	80 ± 4	34 ± 4	7.41 ± .03	95 ± 1	81 ± 1
1 hr postoperative	16	24 ± 3	831 ± 72	83 ± 5	26 ± 2	7.40 ± .02	95 ± 1	80 ± 1
1–12 days postoperative	4*	—	—	81 ± 6	27 ± 1	7.45 ± .01	95 ± 1	76 ± 5
2–4 mos. postoperative	9*	24 ± 1	610 ± 76	72 ± 4	39 ± 8	7.39 ± .03	94 ± 2	78 ± 5
4–24 mos. postoperative	7*	25 ± 2	757 ± 69	82 ± 6	37 ± 4	7.40 ± .02	95 ± 2	78 ± 6
24–44 mos. postoperative	3*	—	—	74 ± 6	36 ± 4	7.38 ± .02	94 ± 2	80 ± 2

Values are expressed as means ± standard error of determinations made while dogs were ventilated with room air.
* Most dogs were studied on more than one occasion.

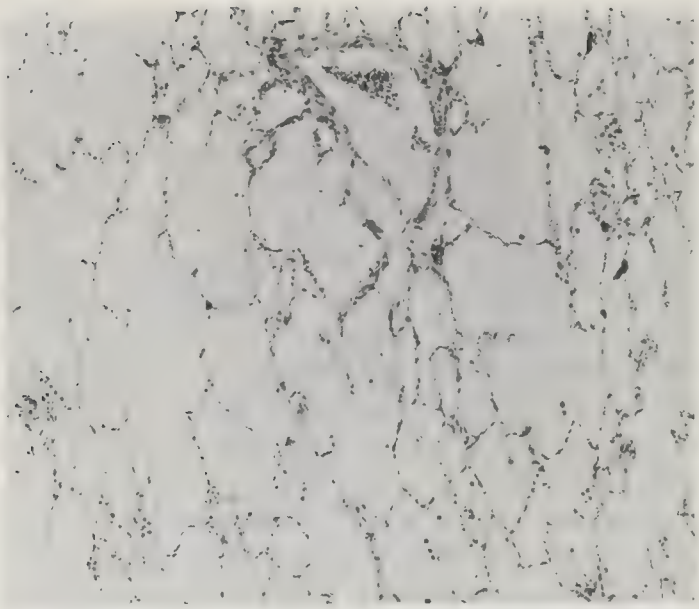


FIGURE 1.—Photomicrograph of allografted left lung 53 days after transplantation and right pulmonary artery ligation. Minimal round cell infiltration is the only abnormality present in this and multiple other sections from this lung. (Hematoxylin and eosin, x100.)

rectly proportional to the increased blood flow, indicating that the vascular resistance of the transplanted lung was not fixed and could decrease normally when its blood flow was augmented. This normal characteristic of vascular resistance in the autografted lung persisted throughout the period of observation, as indicated by the constancy of pulmonary artery pressure and measurements of left lung vascular resistance (Table II).

All blood gas values measured two to 44 months after operation were within the range of normal for lightly anesthetized dogs breathing room air. Detailed values are presented in Table II and were not significantly different from values determined for these parameters preoperatively or in normal dogs in which both lungs are functioning normally.

Pulmonary Angiography

At all times after operation, up to two months in dogs with allografts and up to 24 months in dogs with autografts, pulmonary angiography in all recipient dogs showed that the entire pulmonary blood flow traversed the transplant, confirming that it alone provided pulmonary function. In one autografted dog, an angiogram taken two months after operation revealed that the diameters of the main and left pulmonary arteries had increased significantly when compared with a preoperative angiogram from the same dog (Figure 2). Pulmonary angiograms of all other long-term survivors revealed the pulmonary arterial, capillary, and venous circulations to be quantitatively and qualitatively normal at all times up to two years except for moderate, 25 to 75 per cent, dilatation of the first, second, and third order arteries (Figures 3 and 4). Despite this dilatation, no anastomotic defects were identified in any of the long-term survivors.

Microscopic Morphology

Histologic material from the autografted lungs was available from four dogs that died during the first postoperative week, three dogs that died from one to four weeks after operation, and six dogs that died from three to 30 months after operation. The histologic findings

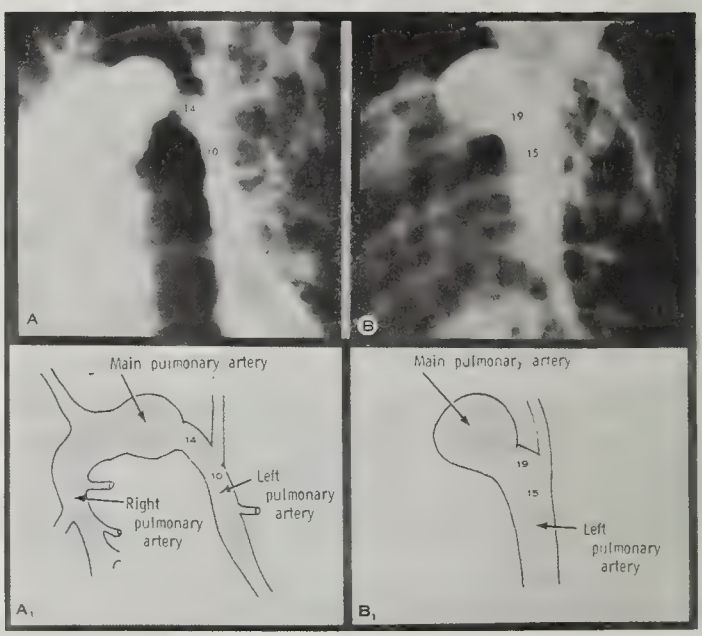


FIGURE 2.— Equivalent views of preoperative and postoperative angiograms of one dog. Both angiograms are in the right posterior oblique projection. A, The preoperative angiogram. B, Angiogram performed two months after operation. A_1 and B_1, Tracings from the original roentgenograms. The numbers on the roentgenograms and the tracings represent the diameters, in millimeters, of the vessels at that site as measured on the original films.



Figure 3.—Right posterior oblique projection of arterial phase of an angiogram performed 17 months after autotransplantation of the left lung and ligation of the right pulmonary artery. Notice that there is no flow through the right pulmonary artery and that there is moderate dilatation of the left main pulmonary artery and its major branches. The site of arterial anastomosis cannot be identified.

in dogs which died within the first month have been described previously.[5] Although many lesions were present in some of the transplants of dogs which died during this period, many showed no structural abnormality, and examples of histologically normal areas could be found in the transplanted lungs of dogs which died at all times during the first postoperative month.

In the two dogs that died four and 12 months after operation, the autotransplanted lung was grossly and microscopically normal (Figure 5) except for focal atelectasis. Two dogs that died three and six months after operation had extensive pneumonia in the right lung with areas of infarction. The transplants in these dogs were normal except for traces of pulmonary edema. The dogs that died of unknown causes after 19 and 30 months had grossly normal lungs bilaterally. Microscopically, the transplanted lungs were normal except for minimal congestion (Figure 6).

DISCUSSION

The present results show that with meticulous surgical technique it is possible to achieve survival after left lung transplantation and immediate right pulmonary artery ligation in approximately fifty per cent of dogs subjected to



FIGURE 4.—Representative views of the arterial, capillary, and venous phases of an angiogram performed on a dog 22 months after autotransplantation of the left lung and ligation of the right pulmonary artery. All views are in the right posterior oblique projection. Except for dilatation of the major arteries of the left lung, no abnormalities are present. No contrast agent entered the right pulmonary artery.

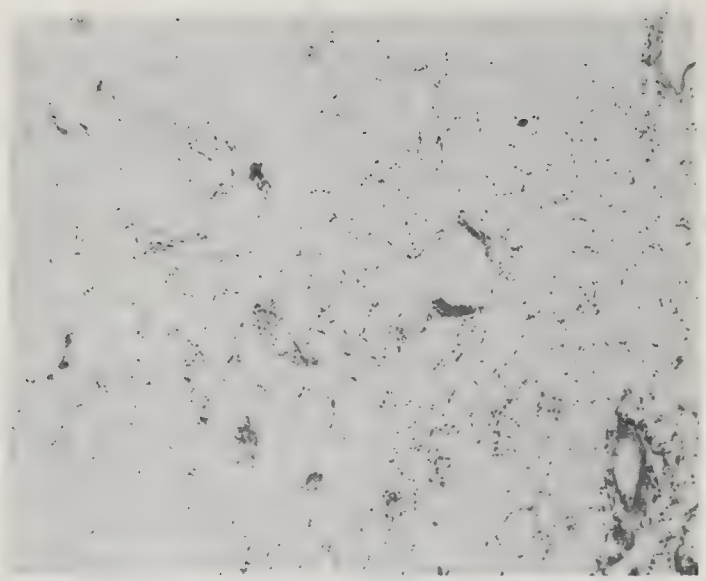


FIGURE 5.—Photomicrograph from the transplanted lung of a dog that died 14 months after autotransplantation of the left lung and ligation of the right pulmonary artery. No significant abnormalities are evident. (Hematoxylin and eosin, x60.)

the procedure. The ability to accomplish this has significance in two regards. First, it supports the use of single lung transplantation in patients with respiratory insufficiency and vascular disease in both lungs. Second, it provides an experimental model for studying the efficacy of immunosuppressive methods and preservation techniques intended for use in lung transplantation.

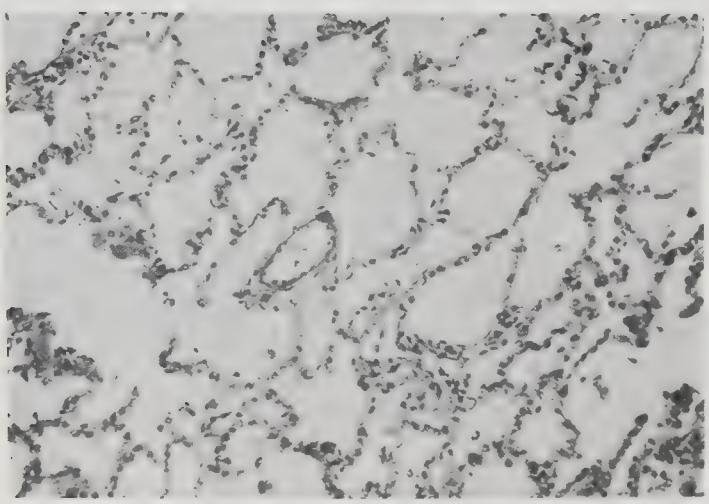


FIGURE 6.—Photomicrograph of the transplanted lung of a dog that died of unknown causes 30 months after autotransplantation of the left lung and right pulmonary artery ligation. Except for minimal congestion, this lung is normal. (Hematoxylin and eosin, x160.)

After our initial descriptions of this model,[4,5] many groups found it difficult to obtain survival with comparable preparations. Some of these investigators also found it difficult to reproduce our gradient measurements at the pulmonary arterial anastomosis and expressed doubt about the importance we attributed to the distensible arterial anastomosis. However, the studies by Noirclerc, Daicoff, and their colleagues proved that even minor constrictions in the left pulmonary artery can, with right pulmonary artery ligation, cause substantial pressure gradients to be generated in the left pulmonary artery.[9,10] And recently, Alican, Ebert, and their associates were able to achieve a high percentage of chronic survivors in dogs that had undergone left lung autotransplantation and immediate contralateral pulmonary artery ligation.[11,12] These authors also stressed the importance of meticulous surgical technique; and although they did not utilize a vein patch or spatulation, they did achieve distensible arterial anastomoses and low pulmonary artery pressures after operation by taking small careful externally placed suture bites in the arterial wall. The importance of distensibility of the arterial anastomoses has now been demonstrated conclusively by the generalized pulmonary arterial widening without anastomotic narrowing seen on postoperative angiography as shown in Figure 2 and confirmed by Alican et al. in dogs [11] and Joseph and Morton in baboons.[13]

Although we originally used a vein patch angioplasty to achieve distensible arterial anastomoses, we no longer believe that this technically difficult maneuver is essential. We and Alican and his colleagues have successfully utilized simpler albeit exacting techniques to fashion nonconstricting transplant arterial anastomoses.[7,11] Whatever technique is employed, we continue to believe that maintenance of distensibility of the large pulmonary arteries including the site of anastomosis is essential to permit normal vasodilation and survival after lung transplantation with immediate ligation of the contralateral pulmonary artery. Comparable technical care is probably not essential to success in human lung transplantation because of the larger size of the structures, because

some blood flow to the opposite lung will probably be maintained, and because of prior right ventricular hypertrophy. It is, of course, also necessary to avoid technical errors in both the bronchial and venous anastomoses in order to obtain animals that can survive solely on the function of a single transplanted lung. Technical details which have facilitated these important aspects of the procedure have been previously detailed by our group[7] and by Alican and his coworkers.[11]

The present results show that 10 per cent of allografted lungs, under treatment with azathioprine, antilymphocyte serum and corticosteroids, could provide total pulmonary function for more than one month. However, all allograft recipients were dead 100 days after operation. This is in contrast to a 50 per cent three month survival after autotransplantation and contralateral pulmonary artery ligation. Clearly, the functional defects produced by rejection or the toxicity of the immunosuppressive agents were a major cause of mortality. Since many allografted dogs died with minimal changes in their allograft, we believe that both factors played a role. Obviously better immunosuppression is needed.

The blood gas and hemodynamic data in the present report provide information concerning the late respiratory and vascular function of lung transplants chronically charged with providing total pulmonary function. Clearly such transplanted lungs alone can maintain normal arterial and venous partial pressures of oxygen and carbon dioxide and pH at all times up to 44 months after operation (Table II). In addition, these transplanted lungs were able to vasodilate with increased blood flow so that the pulmonary artery pressure did not rise more than it does in normal dogs subjected to simple right pulmonary artery ligation (Table II). This is an important observation since many other workers have suggested that transplanted lungs have a fixed high vascular resistance and are incapable of vasodilating normally with increased pulmonary blood flows. Furthermore as shown in Table II, no late or progressive rise in pulmonary artery pressure or in the vascular resistance of the transplanted lungs occurred for periods up to two years after operation despite their increased blood flow. This is important since several other investigators have noted a progressive rise in pulmonary artery pressure after lung transplantation and delayed ablation of the function of the opposite lung. It is still unclear what would happen to vascular resistance in these lungs if cardiac output was increased further, although Ebert has presented evidence that transplant vascular resistance is higher than normal with very high cardiac outputs up to 42 days after operation.[12]

The sustained normal respiratory and vascular function of these transplanted lungs is consistent with the normal angiographic and microscopic structure that we observed up to 2½ years after they were charged with providing total pulmonary function. Many workers have claimed that autografted lungs, with time, develop marked histologic abnormalities characterized by fibrosis, foci of bronchopneumonia, and thickening of alveolar walls. Review of the sections from the transplanted lungs in our dogs that died from 2–30 months after operation revealed widespread areas of completely normal lung in all animals. We, therefore, believe that transplanted lungs do not necessarily develop late pathologic lesions, and the changes observed by other workers are misleading and probably related to endemic disease in the recipient animals or to the studies themselves. This conclusion, which is supported by the work of Lempert and his colleagues,[14] is most important since it means that lung grafts can, if rejection is controlled, maintain morphologic integrity for protracted periods and probably indefinitely.

CONCLUSIONS

We believe that we have shown that a single transplanted lung can provide total pulmonary function while carrying the entire cardiac output in dogs, if the arterial anastomosis is distensible and if all other technical details are perfect. This functional adequacy can be achieved in a high percentage of autografts and is well maintained for over 3½ years. With autografts there is no deterioration in respiratory function, vascular function, or pulmonary microscopic morphology for at least 2–3 years.

With allografts an occasional animal will survive more than 2 months with present immunosuppression. Failure to achieve longer survivals with allografted lungs is an indication of imperfections and/or toxicity of present immunosuppression. Thus, the present work provides considerable support for the ultimate feasibility of single lung transplantation in patients with bilateral pulmonary disease. It also provides a laboratory model for studying the improved methods of immunosuppression that will be necessary to improve the poor results thus far achieved with therapeutic human lung allotransplantation.

REFERENCES

1. Duvoisin, G. E., Fowler, W. S. Payne, W. S., and Ellis, F. H. Reimplantation of the dog lung with survival after contralateral pneumonectomy. Surg. Forum 15:173–175, 1964.
2. Faber, L. P., Pedeira, A. L. S., Pevsner, P. H., and Beattie, E. J. The immediate and long-term physiologic function of bilateral reimplanted lungs. J. Thorac. Cardiovasc. Surg. 50:761–768, 1965.
3. Haglin, J., and Arnar, O. Physiologic studies of the baboon living on only the reimplanted lung. Surg. Forum 15:175–176, 1964.
4. Veith, F. J., and Richards, K. Mechanism and prevention of fixed high vascular resistance in autografted and allografted lungs. Science 163:699–701, 1969.
5. Veith, F. J., and Richards, K. Lung transplantation with simultaneous contralateral pulmonary artery ligation. Surg. Gynec. Obstet. 129:768–774, 1969.
6. Veith, F. J., Richards, K., and Lalezari, P. Protracted survival after homotransplantation of the lung and simultaneous contralateral pulmonary artery ligation. J. Thorac. Cardiovasc. Surg., 58:829–836, 1969.
7. Veith, F. J., and Richards, K. Improved technic for canine lung transplantation. Ann. Surg. 171:533–558, 1970.
8. Veith, F. J., Siegelman, S.S., and Dougherty, J. C. Long term survival after lung autotransplantation and immediate contralateral pulmonary artery ligation, Surg. Gynec. Obstet. 133:425–432, 1971.
9. Donnarel, G., Lebreuil, G., Sommer, A., Chauvin, G., Appaix, M., Courbil, J., Malmejac, C., and Noirclerc, M. Factors in the origin of pulmonary hypertension observed after lung transplantation. Lyon Med. 5:51–62, 1969.
10. Daicoff, G. R., Allen, P. D., and Streck, C. J. Pulmonary vascular resistance following lung reimplantation and transplantation. Ann. Thorac. Surg. 9:569–579, 1970.
11. Alican F., Cayirli, M., Isin, E., and Hardy, J. D. Left lung replantation with immediate right pulmonary artery ligation. Ann. Surg. 174:34–43, 1971.
12. Ebert, P. A., and Hudson, B. H. Pulmonary hemodynamics following lung autotransplantation. J. Thorac. Cardiovasc. Surg. 62:188–192, 1971.
13. Joseph, W. L., and Morton, D. L. Long-term survival in the immediately functioning transplanted primate lung. Ann. Thorac. Surg. 11:442–449, 1971.
14. Lempert, N., Price, A., and Blumenstock, D. A. Bilateral canine pulmonary autotransplantation; long-term survival. In *Current Topics in Surgical Research*. Vol. III. New York: Academic Press, Inc. pp. 271–283, 1971.

DISCUSSION

Dr. Veith: I think we will pause at this point for questions and discussion.

Gerald Moss, Rensselaer Polytechnic Institute, Troy, New York: When you measured the resistance of that one lung before and after the transplant, was the left lung receiving less than 50 per cent of the total cardiac output or was there temporary occlusion of the right pulmonary artery?

Dr. Veith: We just measured main pulmonary arterial pressure and cardiac output and assumed 45 per cent of it was going to the left lung.

Dr. Moss: You would expect with the total cardiac output going through that left lung normally, the resistance would drop to approximately half, which is a normal effect, because your pulmonary artery pressure shouldn't rise.

Dr. Veith: You're correct. I didn't put in the other obvious control—normal animals that undergo right pulmonary artery ligation. In essence, the left lung in the dog is smaller than the left lung in other experimental animals and in man, and when you measure pulmonary artery pressure after right pulmonary artery ligation in a normal dog, this is the approximate range of the pressure change. In other words, after the operative procedures the animal's total pulmonary vascular resistance goes up, which would be true in any animal subjected to anesthesia and thoracotomy. More importantly, the total pulmonary vascular resistance falls after the transplant, just as it would in a normal animal.

DR. MOSS: I meant, that the initial resistance you had, which appeared to be the same as when you started, in actuality was higher than it would have been in the normal lung because normal resistance would have fallen under the increased flow.

DR. VEITH: Right. I understand what you're saying. The point is that the animal that received a transplant behaved identically to the normal dog.

QUESTIONER (unidentified): Do you really need to use Imuran?

DR. VEITH: We've been using a combination of Imuran, ALS, and steroids, and although our figures are not statistically significant, it appears that the animals who received no prednisone and lower doses of Imuran and ALS did the best. It may just have been luck and a combination of other factors. I'm intrigued by your observation that you don't need the Imuran.

MIKE PETER, Harbor General Hospital: What kind of problems are you having with infection? Do you recognize and treat it? What per cent mortality is related to infection?

DR. VEITH: I'd have to look at our exact figures. A moderate number of the animals develop pneumonia, which is seen histologically in the allografted lungs. Pneumonia often occurs in conjunction with rejection, and I'm not sure which comes first. I actually think that the rejection comes first, and we have a number of studies on our own patients and on histologic sections from other patients that point to the unusual forms rejection may take in the lung. This is a controversial issue but it appears that in our patients, at least, rejection is identifiable and reversible, and has now been distinguished from infection with reasonable accuracy in two patients. It also appears that what one sees on the x-ray as either infection or rejection is most often rejection.

DR. PETER: Can you reverse rejection in dogs?

DR. VEITH: The appropriate study is now in progress, but we certainly think we can do it in man.

CHAPTER V

HEMATOLOGY

Blood Grouping Systems in Dogs, Cattle and Sheep

Factors Regulating the Production of Coagulation Activities in Perfused Organs

Hemostasis in the Animal Kingdom

Blood Groups of Apes and Monkeys: Human and Simian Types

Sheep Blood Coagulation Values

Experimental Approaches to the Study of Vitamin K

Summary of Contribution of Hemophilic Dogs to Knowledge and Human Welfare

A Survey of Blood Groups in Several Species of Large Animals Used in Medical Research

BLOOD GROUPING SYSTEMS IN DOGS, CATTLE AND SHEEP

E. B. Black and J. M. Stengle*

During the past thirty years complexes of antigens have been discovered on the erythrocytes of dogs, cattle, and sheep which have led to the development of blood grouping systems for each species comparable to, although in many respects more intricate than, that in the human. The first major blood group in cattle, blood group A, was discovered in 1940. Since then the number of known bovine blood groups has been expanded to eleven, including the B system, which is probably the most complex blood group in any species and the J system with the intriguing relationship of "J substance" to bovine serum and erythrocytes. Seven major blood group systems are now recognized in sheep including the M-L system. The antigens of this system may be biochemical entities which play an important role in biological membrane function. The canine red cell antigenic system contains seven major blood groups. Transfusion reactions in A negative dogs immunized with type A_1 erythrocytes provide an interesting comparison with hemolytic transfusion reactions in the human. Although of little significance from a clinical veterinary standpoint, ovine, canine, and bovine blood group analysis presents a major challenge to the imagination and technology of the immunohematologist, the geneticist, and biochemist, and the evolutionist.

INTRODUCTION

During the past thirty years complexes of antigens have been discovered on the erythrocytes of dogs, cattle and sheep which has led to the development of blood grouping systems comparable to, although in some respects more intricate than that in the human. Although of little significance from a clinical veterinary standpoint, canine, bovine, and ovine blood group analysis present a major challenge to the imagination and technology of the immunohematologist, the geneticist, the biochemist, and the evolutionist.

* National Blood Resource Program, National Institutes of Health, Bethesda, Maryland.

SHEEP

Seven major antigenic groups have been identified on the erythrocytes of sheep: R-O, X-Z, A, D, M-L, C, and B. The R-O system was the first discovered. Our current knowledge of that system is founded upon the early work of Bialosuknia and Kaczkowski[1-4] in the mid-1920's and that of Andersen[5] in the late 1930's. These workers demonstrated that the red cells of some sheep were agglutinated or lysed by naturally occurring isoantibodies from ovine serum and that the red cells of other sheep were not. The former group is now recognized as R and the latter as O.

In the early 1950's Stormont[6] isolated naturally occurring antibodies from bovine serum which reacted with group O sheep erythrocytes. In tests of over 100 bloods with anti-R anti-O reagents, Stormont observed mutually exclusive R and O reactions indicating that O is truly recessive to R and is not expressed in the heterozygous condition. In 1954 Rendel[7] et al. discovered a third phenotypic red cell in sheep which was lysed by neither anti-R nor anti-O. This phenotype is called i. At least two genetic loci play a part in the control of the R, O, and i phenotypes. Where r° represents the gene which when homozygous gives rise to the O phenotype, the genotype of sheep of group R is $I/- \; R/-$; the genotype of group O is $I/- \; r^\circ r^\circ$. The recessive genotype ii is epistatic to both R and O (Figure 1). In 1958 Rasmusen discovered an i lamb from an $O \times O$ mating, supporting the hypothesis that ii is epistatic to O. Anti-R antibodies are frequently encountered as a natural component of the serum of R negative sheep. Anti-R antibodies were found in all of 20 group O sheep studied by Rendel et al.; however, the sera of only two out of 23 group i sheep were shown to contain Anti-R. Sheep

Gene combinations	Substances in saliva*		Substances in serum, acquired by red blood cells	
	O	R	O	R
I/-R/-	+	+	–	+
I/-r^0r^0	+	–	+	–
ii	–	–	–	–

* As indicated by inhibition tests.

FIGURE 1.—Genotypes and phenotypes in the R-O system[12]

anti-R sera are often variable in their reaction with R erythrocytes. For this reason most screening for the R antigen is done with bovine anti-J antiserum which cross-reacts well and is not as variable in its reactions with the sheep R-antigen.

The erythrocytes of newborn lambs are R-negative and O-negative. However, depending upon the lamb's genetic complement, soluble R or O substance can be detected in the serum by inhibition tests. Studies by Rendel[8] have shown that the erythrocytes of newborn lambs with R substance in their serum became R-positive in an average of 16.4 days after birth and that lambs with detectable O substance show O positive erythrocytes in an average of 28.4 days after birth. Anti-R is not detectable in the serum of newborn lambs before suckling. It is detectable in group O lambs during suckling if the dam is group O. However, these passively acquired antibodies disappear at from 10 to 26 days of age, and no actively produced anti-R is detectable before the lamb is 5 to 7 months of age.

In R positive adult sheep the plasma contains soluble R substance and the plasma of O positive sheep contains soluble O substance. In group R sheep, R substance is not only detectable in the erythrocytes and in the serum, it has also been demonstrated in the milk, saliva, and seminal fluid. O substance has been detected in the milk, plasma and semial fluid of group O sheep, and also in the seminal fluid and plasma of group R sheep. Neither substance has been detected in significant amounts in the body fluids of group i sheep. Rendel[8] has postulated that the I gene functions in a capacity similar to the secretor gene in man who secretes Le^b substance in his saliva. Rendel et al.[7] have shown that group O erythrocytes incubated in serum from a group R sheep became R positive and can be lysed by both anti-R and anti-O sera. Likewise, group i erythrocytes became O positive when incubated with serum from group O sheep. Thus the R and O antigens are apparently produced by cells outside of the hematopoietic system and are acquired as a coating substance by the sheep erythrocytes.

The R and O antigens of sheep have been demonstrated to be serologically related to erythrocyte antigens in other species. For example, Amzel et al.[9] have shown a serologic relationship between R of sheep and A of man. Neimann-Sorensen et al.[10] have studied the serologic relationship of A of man, R of sheep and J of cattle. The O substance of sheep is related to the H substance of man: saliva from human secretors inhibits the O anti-sheep O reaction while saliva from human non-secretors does not.[10]

The X-Z system was the second major blood group discovered in sheep. It is a two allele system with three recognized phenotypes: X, XZ, and Z. It was first described by Rasmusen[11] using as a reagent heteroimmune antisera perpared by immunization of rabbits with sheep red cells. The heteroimmune antiserum lysed X-positive sheep erythrocytes in the presence of rabbit or guinea pig complement. When solutions of anti-X antiserum were adsorbed with sheep erythrocytes from ten individuals, it was noted that the erythrocytes from one individual failed to adsorb all hemolysins. When the red cells of the nine individuals which has shown complete adsorption were incubated in the presence of complement in the single solution where adsorption was incomplete, lysis occurred in seven cases, but the erythrocytes of two individuals remained unlysed. This was interpreted as evidence that genetic dosage was involved in the reactions. The hemolysins remaining in the solution where adsorption was incomplete might be in sufficient quantity to lyse red cells from sheep homozygous for factor X but not to lyse cells from sheep heterozygous for factor X. The search for a complementary blood factor Z was begun. Anti-Z antiserum was first prepared by

immunizing rabbits with red cells from various species including springbok, blesbok, eland, pranghorn antelope, and bison. The antiserum against springbok red cells most consistently provided anti-Z antibodies, which remained in the serum after adsorption with type X erythrocytes. In over 1,000 sheep typed by Rasmusen,[11] all were either X, XZ or Z. In 40 sheep tested, 29 were X, 10 were XZ, and 1 was Z.

By 1960, the A, C, D, M-L, and B systems had been discovered using reagents prepared from the sera of sheep, goats, cattle or rabbits immunized with sheep erythrocytes. The B system is very complex and seems to be homologous to the bovine B system which will be discussed later. The D system with its two phenogroups is the only system whose test reagent has agglutinating rather than hemolysing properties. The A system consists of two phenogroups, A and "—" (no A) and the C system has three phenotypes C, C_x, and "—" (no C).[12]

The M-L system and its relationship to the cation content of the sheep erythrocyte has become an important area of research which may shed light upon some of the basic mechanisms of biologic membrane function. The presence of the M antigen is associated with a high red cell potassium content (HK); whereas, the L antigen is present in red cells with a low potassium content (LK).[13-15] In the heterozygous (ML) condition in this two allele system, the LK state is dominant. HK erythrocytes differ from those of the LK type in both active and passive transport of sodium and potassium. Active transport of sodium and potassium is four to seven times greater in HK than in LK erythrocytes. Likewise, the Ouabain inhibited sodium and potassium stimulated ATPase has been shown to be more active in HK than in LK cells. When LK cells (blood type LL) are incubated in isoimmune anti-Lantisera prepared by immunizing HK (blood type MM) sheep with red cells from an LK (blood type ML) sheep, the potassium influx in the LK cells increases six-fold. In contrast, when HK red cells are incubated in either anti-LM or anti-MM antisera, the sodium-potassium transport characteristics of the cell membrane are not altered. An experiment by Lauf, Rasmusen, Tosteson, et al.[15-17] showed a 5.5-fold increase in potassium pump influx in LK cells exposed to anti-Lantiserum. When the concentration of anti-L was reduced by successive dilutions, a two-fold increase in the potassium influx was observed at a concentration of anti-L antiserum $\frac{1}{20}$ that of the undiluted antiserum (Figure 2).

Ouabain is known to inhibit the potassium pump. It is thought that one molecule of Ouabain blocks one pump site and that the activity of the pump is inversely proportional to the number of Ouabain molecules bound to the cell membrane.[18] Therefore, the number of potassium pump sites can be estimated if the number of Ouabain molecules bound to the membrane is known. In experiments to determine the effect of anti-L antisera upon the number of pump sites, Ouabain binding was measured in LK erythrocytes incubated in anti-L antiserum and in non-immune serum (NK-NI). Ouabain binding to the erythrocytes incubated an anti-L was approximately twice that seen when non-immune serum was used (Figure 3). These results suggest that anti-L antiserum acts by approximately doubling the number of active transport sites on the red cell membrane. However, the kinetic properties of the sites must also be altered since a 4–7 fold increase in the concentration of intracellular potassium is observed.

Snyder, Rasmusen, and Lauf[19] have charac-

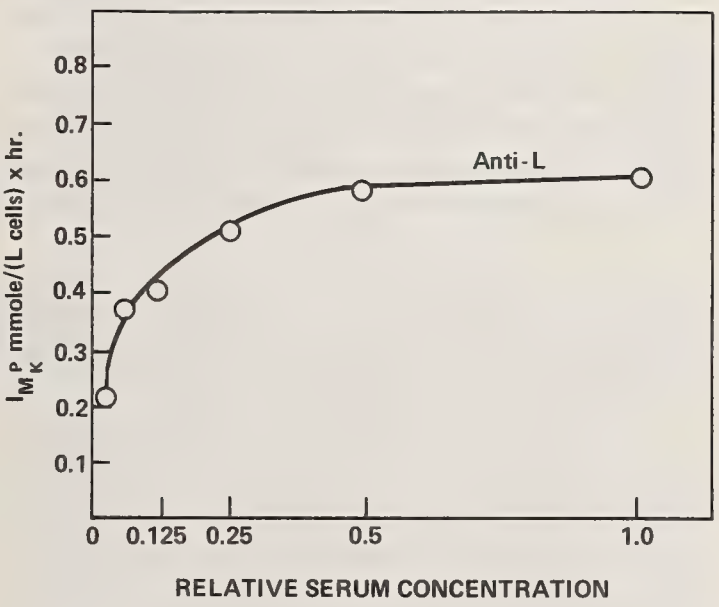


FIGURE 2.—Effect of varying concentrations of anti-L-antiserum on the active transport of potassium in LK erythrocytes[17]

Experiment	Serum	T-ouabain (M)/ preincubation	K^+-pump flux [mM/(L cells) x hr]	Inhibition (%)	Molecules T-ouabain/ cell	Pump sites/ cell
E 84	Anti-L	0	0.28			
		10^{-7}	0.12	57	70	122
	HK NI	0	0.11			
		10^{-7}	0.065	41	40	97
E 86	Anti-L	0	0.26			
		10^{-7}	0.076	71	45	63
	HK NII	0	0.001			
		10^{-7}	<0	71	21	30*
E 88	Anti-L	0	0.38			
		10^{-7}	0.25	37	30	82
	HK NI	0	0.094			
		10^{-7}	0.059	37	10	27

* Since no K^+-pump flux was measured in this part of exp. 86, the degree of inhibition by 10^{-7}M T-ouabain could not be estimated. Therefore, the number of pump sites was computed on the basis of 71% K^+-pump flux inhibition found in presence of anti-L.

FIGURE 3.—Effect of L-antiserum on the number of ouabain molecules bound per LK sheep red cell[17]

terized the anti-Lantibody as an IgG immunoglobulin. The same investigators have shown that the $F(ab)_2$ antibody fragment alone is capable of stimulating potassium influx in LK cells. The physical and chemical characteristics of the M and L antigens are still unknown.

CATTLE

At least 11 major blood grouping systems have been identified in cattle: A, B, C, F-V, J, L, M, N, S, Z, and $R^1 - S^1$ (Figure 4). Some laboratories report more major bovine blood groups than those listed here. However, with the exception of N, these are the most widely recognized. All bovine blood typing has been done with lytic techniques since bovine erythrocytes tend not to agglutinate even when they have been incubated with multiple sensitizing doses of blood typing antibodies. Rabbit and guinea pig complement are used in routine typing of bovine blood. At present over 100 blood typing reagents are used in analyzing cattle blood groups.

Genetic systems	Year discovered	Number of blood factors	Number of phenogroups	Number of distinguishable phenotypes
A	1944	5	10	13
B	1940	50	300 +	15,000?
C	1941	10	35 +	200?
F–V	1943	5	4	9
J	1942	2	4	4
L	1947	1	2	2
M	1958	2	3	3
N	1960	1	2	2
S	1943	6	5	12
Z	1941	1	2	3
R′–S′	1960	2	2	3

FIGURE 4.—Major blood groups in cattle[23]

The B system was the first bovine blood group to be extensively studied. It has become one of the most complex blood groups known in any species. Group B was first recognized as a multiple allelic system in 1940 by Stormont who observed that bovine blood factor K appeared only in the presence of factors B and G. He proposed that the observed phenotypes BGK, BG, B, G, and no-BGK were controlled by multiple alleles which he designated b^{BGK}, b^{BG}, b^{B}, b^{G}, and b^{-}. Here the superscripts BGK, BG, B, G, and "—" are referred to as phenogroups of the B system. It soon became apparent that many other blood factors were in fact part of the B system[20-22] including such phenogroups as $BGKO_2Y_1A'E'_3G''K'Y'B'O'$, $O_1T_1E'_3F'$, K′, PQE'_1, GO_xO', and $BGKO_xY_1A'$. During the past 30 years more than 300 phenogroups have been recognized in the B system. There are more than 45,000 possible diploid combinations of B phenogroups.[23] How many of these would produce serologically distinguishable phenotypes is anybody's guess. The range of possibilities continues to grow as new alleles are discovered, some probably appearing as a result of spontaneous mutation.

The J antigen was discovered in 1942 by Ferguson, Stormont, and Irwin[21] working with naturally occurring antibodies from the blood of what were later to be classified as J negative cattle. Soon thereafter a soluble substance was found in the serum of J positive cattle which inhibited the reaction of cellular J substance with anti-J antisera. On the basis of these observations, Stormont proposed that J substance is primarily a soluble antigenic entity elaborated in J positive cattle by cells outside the circulatory system. J substance has been found in areas outside of the circulation including the saliva, gastric mucosa, and urine. It is adsorbed by the erythrocytes from the serum. The ability of cattle erythrocytes to adsorb J substance was demonstrated when erythrocytes from J negative individuals were incubated in J positive serum. In the presence of the J positive serum, J negative cells became serologically J positive. Stone and Irwin[25] later discovered cattle with eryth-

rocytes which were J negative but which had serologically detectable J substance in the serum. Thus three phenotypes were identified in the J system: J^{cs} with J substance present on red cells and in the serum; J^{s} with J substance in the serum but not detectable on the red cells by the usual typing procedures; and j^{a} with no detectable J substance.

Although J substance is present in the sera of both J^{s} and J^{cs} individuals, its concentration (measured in units of J where one unit is equal to the reciprocal of the highest dilution of strongly reactive J reagent giving 50% or greater hemolysis within 4 hours[25]) is usually much higher in the serum of J^{cs} cattle than in the serum of J^{s} cattle. The initial hypothesis suggested that the higher serum concentration was necessary for J substance to appear on the erythrocytes.[25] However, exceptions to this were noted. Of the 194 animals typed by Stone, et al., (Figure 5) 70 were of the class J^{s}. Of the J^{s} individuals only 4 had serum J levels greater than 16 units. Of 124 J^{cs} cattle 102 had serum J levels above 16 units, 15 had serum J levels of exactly 16 units, and 7 had serum J levels of less than 16 units. If 16 units of serum J substance represented a threshold above which erythrocytes tended to adsorb J substance and below which they tended not to adsorb J substance, then in this study the 4 J^{s} individuals

RELATIONSHIP BETWEEN UNITS OF J SUBSTANCE IN THE SERUM AND ON THE CELLS OF 194 DIFFERENT CATTLE BLOODS

UNITS OF J SUBSTANCE ON THE CELLS	0	<2	2	4	8	16	32	64	128	256	>256
>256											
256						1	1				
128						2	1	2	14	6	2
64					1	3	7	26	8	8	1
32				1	3	2	4	4	5	3	
16						1	1	2	1		
8						2	1		1		
4						1		2	1		
2											
<2				1	1	3	1				
0		19	13	9	17	8	3	1			

UNITS OF J SUBSTANCE IN THE SERUM

FIGURE 5.—Relationship between units of J substance in the serum and on the cells of 194 different cattle bloods[25]

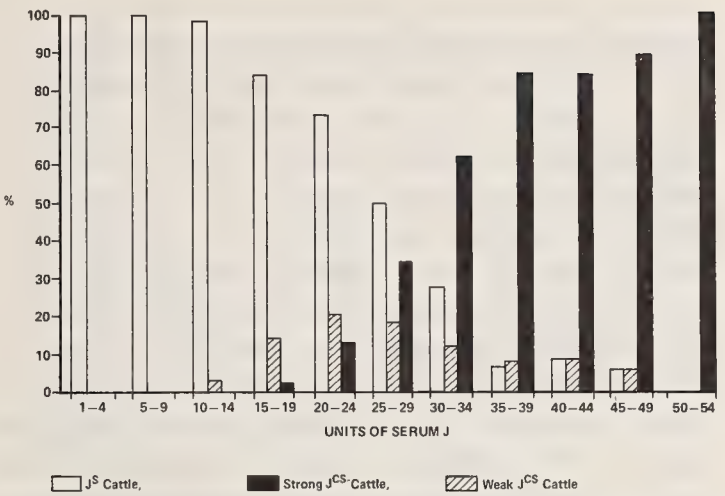


FIGURE 6.—Relationship of serum and cellular J of 966 J^{cs} and J^{s} cattle over one month of age[26]

with serum J greater than 16 units and the 7 J^{cs} individuals with serum J less than 16 units represented exceptions to the rule. In larger samples of cattle, 2% showed J^{s} red cells in the presence of greater than "threshold" concentration of serum J and about 4% showed J^{cs} red cells in the presence of a below "threshold" concentration of serum J. Thus it appeared that other factors in addition to the serum J concentration might be operative in determining whether or not the red cells acquired J substance.

Further investigations by Patel[26,27] confirmed the assumption that serum concentration of J substance is one factor, but not the only factor, playing a role in the acquisition of J substance by cattle red cells. The relationship between the concentrations of serum and erythrocytic J substance was analyzed in 966 cattle (Figure 6). The J^{cs} phenotype was divided into strong J^{cs} (greater than 30 units of cellular J substance) and weak J^{cs} (less than 30 units of cellular J). The distributions of strong J^{cs}, weak J^{cs}, and J^{s} were analyzed as a function of J concentration in the serum. Again there was evidence that there is a direct relationship between serum J concentration and the adsorption of J substance to the erythrocytes. In Patel's data a threshold range of about 25–34 units of serum J substance existed above which 86% of the cattle had red cells which had acquired enough J substance to be J positive by routine tests and below which 86% were J negative by

routine tests, and therefore fell into the J^s class. Still, however, obvious exceptions to any "threshold hypothesis" were noted. Two-thirds of the cattle within the threshold range were type J^{cs} and one-third were type J^s. Fourteen percent of the cattle serum J concentrations above 34 units had erythrocytes which reacted not at all or only very slightly with anti-J antisera. These cells were classified as J^s or weak J^{cs}. Fourteen percent of cattle with serum J concentrations below 24 units were strongly reactive with anti-J antisera and were classed as strong J^{cs}. Pedigree studies suggested that each of the exceptional phenotypes was inherited.

In another series of experiments Patel[26,27] incubated washed cattle erythrocytes of all types in sera from J^s, J^{cs} (strong), and J^{cs} (weak) cattle. Red cells incubated in J^s serum even when J was of high concentration, did not adsorb J substance. Likewise, red cells incubated in J^{cs} (weak) serum did not adsorb J substance even though in some cases the serum J concentration was higher than that seen in some J^{cs} (strong) sera. However, all J^s and J^{cs} (weak) cells incubated in sera from J^{cs} (strong) cattle acquired J substance even in those exceptional cases where the serum concentration of J was below threshold.

In still another study J^a cells were incubated in serum containing 40 units of J substance from J^{cs} (weak) cattle. The j^a cells remained J negative. However, when the same cells were incubated in serum containing 40 units of J substance from J^{cs} (strong) cattle, J substance was adsorbed and the cells became J positive. Thus there appears to be a serum factor which inhibits the adsorption of J substance on to the erythrocytes. During the immediate post-natal period, the erythrocytes of all calves are J negative regardless of genotype. The cells of J^{cs} cattle adsorb J substance from the serum and attain adult levels by the fourth week of life. If, however, red cells are removed from a newborn calf of any genotype and are incubated in sera from adult J^{cs} cattle, they adsorb J substance and become J positive.

In summary both the serum concentration of J substance and a heritable serum factor seem to play important roles in determining whether or not red cells adsorb J substance from the serum. The characteristics of the serum-antigen-cell relationship are still under investigation.

The biochemical nature of the soluble J antigen is currently being studied. When J positive bovine serum is fractionated, the total lipid fraction retains haptenic activity in the homologous J system. In contrast the total serum lipid fraction from J negative cattle contains no J haptenic activity. After further fractionation procedures the J haptenic activity remains only in the purified glycosphingolipid[28] fraction leading to the current assumption that in the serum soluble form J substance is a lipoprotein with a glycosphingolipid haptenic component. When low density lipoproteins are isolated from bovine serum by ultracentrifugation, they are found to contain J substance. In other species serum soluble blood antigenic substances such as the Lewis blood group is humans [29] and the A blood group in pigs have been shown to contain important lipid components.

In 1958 Sprague[30] discovered another serum soluble substance, O_c, and demonstrated that it is probably an allele of the J system. O_c was first detected when it was observed that sera from certain J positive or J negative sheep inhibited the hemolysis of type O sheep erythrocytes by antisheep -O- antisera. Also, it was noted that the red cells from cattle with O_c positive serum, although they were not hemolyzed by antisheep -O- antisera, adsorbed antisheep-O and prevented the hemolysis of type O sheep cells by antisheep O in the presence of complement. The four major defined phenotypes of the J system then are: J, JO_c, O_c, and "—". It is certain that many more alleles as yet undefined are also included in the J system and account for the varying distribution and concentrations of bovine J substance.

The A, C, F-V, L, M, N, S, Z, and $R^1 - S^1$ systems have all been discovered by isoimmunization techniques. There are more than 2×10^{12} possible phenotypic combinations of alleles which determine bovine blood types, and the complexity of the system will increase as more alleles and possibly more major blood groups are discovered.

From a practical point of view, our knowl-

edge of bovine blood groups, and for that matter of any animal blood typing system, may provide an insight into evolutionary relationships between species and between breeds within a species. For example, a remarkable similarity exists between the blood grouping systems of cattle and of the American bison with homologies in not less than nine major blood types. On the basis of this observation, Stormont et al.[31] have suggested that domestic cattle and the American Bison be classified as members of the same genus. In Europe, the blood factor Z′, an allele of the bovine A system, occurs in a limited number of breeds of cattle distributed in a pattern from Italy northward through France to southern England. The Z′ allele is absent from Scandanavian[32-34] and German[35] breeds but is seen in the Brahman breed of Asian origin. Not only does the distribution of the Z′ indicate a genetic relationship between several geographically separated breeds of cattle, it may well mark the progress of early man as he migrated from southern Asia to Europe taking his livestock with him.

DOGS

Although the dog has long been the principal research animal in many laboratories, its blood grouping systems have been studied less extensively than those of many other species including sheep and cattle. Seven major canine red cell antigens are recognized: A (subgroups A_1 and A_2) B, C, D, E, F, and G (Figure 7). Each gene when present acts as a dominant factor[36] and, except in the A system, multiple alleles have not been identified. Naturally occurring reciprocal antibodies are encountered only occasionally. As in cattle and sheep most canine blood group discovery has been accomplished using isoimmunization techniques. The first recognition of blood groups in dogs was made by Von Dungern and Hirzfeld[37] almost 62 years ago with a description of four blood types. In 1913 Ottenberg, Kaliski, and Friedman[38] reported the first transfusion reactions in dogs infused with incompatible canine blood. The phenomenon of isoimmunization in dogs infused with incompatible blood was first recognized in 1924 by McEnery, Ivy and Pechous.[39] Their observations, followed by similar observations of Melnick, Burack, and Cowgill[40] and others,[41-50] laid the foundation for the development of the modern canine blood grouping system.

Antigen	No. Dogs Tested	Incidence (%)	Antigen	No. Dogs Tested	Incidence (%)
A	954	62.6	D	764	22.3
Subgroup A_1	332	44.6	E	231	73.1
Subgroup A_2	332	19	F	165	99.4 (one animal negative)
B	867	5.5	G	18	*
C	947	98.4			

FIGURE 7.—Major canine blood group antigens[51]

The canine A blood group, seen in about 63% of randomly sampled dogs, is from a scientific standpoint the most important of the canine blood systems. The A group is unique among canine blood types in that it exists in more than one grade of reactivity[50] and is apparently the only canine blood group which regularly fixes complement.[52] When erythrocytes from several A positive dogs are incubated in separate test tubes with several samples of anti-A antisera in the presence of complement, varying reactions are observed. In some tubes, various degrees of hemolysis occur and the cells which are not hemolyzed are agglutinated. In other tubes A positive cells are agglutinated ranging in degree from strong to weak with no observable hemolysis. In still other tubes red cells are neither agglutinated nor hemolyzed but become markedly reactive in the antiglobulin test.

In 1952 it was suggested by O'Brien that type A cells which did not hemolyze when incubated with anti-A antisera in the presence of complement in fact represented a subgroup of A antigen significantly different from the antigen associated with *in vitro* hemolysis. Soon thereafter, an A positive dog whose red cells produced only a strong agglutination reaction was immunized with type A red cells which reacted with complete hemolysis. An antiserum was produced which reacted only with those type A red cells which were hemolyzed to some extent when incubated with anti-A and complement and not with those which showed agglutination only. The subgroups of A proposed by O'Brien were then serologically distinguishable. Subgroup A_1 included dogs having type A erythro-

cytes which hemolyzed in the presence of antisera and complement, and subgroup A_2 included those dogs having group A erythrocytes which reacted with no hemolysis. Subgroup A_1 cells were non-reactive in the presence of anti-A_2 antiserum and complement.

The system is the canine blood group responsible for most observed transfusion reactions in dogs. In the dog the transfusion reaction is rarely of clinical significance since they usually occur only in a setting of previous sensitization, and when they do occur, although their clinical manifestations may seem severe, death rarely results. Recovery is usually rapid and complete. Transfusion reactions in dogs present some interesting parallels to severe hemolytic transfusion reactions in humans.

In most cases a single transfusion of group A red cells into an unsensitized A negative dog will be tolerated without clinical manifestations. Within a few days anti-A antibodies appear in the serum of the recipient resulting in a shortened life span for the transfused A positive cells. Since, in a clinical veterinary setting, it is rare for a dog to be multiply transfused, initial isosensitization is usually not a problem and typing is often not undertaken in choosing donor blood. However, if the sensitized dog is transfused with type A_1 red cells, a severe transfusion reaction may result characterized by chills, emesis, urinary and fecal incontinence, transient prostration, and fever beginning within a few minutes after the transfusion is initiated.[51,53] Occasionally hives, asthmatic respirations, transient paresis of the hind limbs, and convulsions are seen either during or immediately after acute hemolytic reactions. Hemolysis, often severe, is usually a component of the reaction and is accompanied by hemoglobinuria and hemoglobinemia. The severe signs usually subside rapidly and the dog remains febrile and dehydrated for about 24 hours, after which the animal returns to normal. The renal function remains unimpaired, and, unlike the case in humans, histologic examination of the kidneys reveals none of the stigmata of acute tubular necrosis.[54] An understanding of the reasons for the absence of renal damage in the dog might increase our understanding of the genesis of acute renal failure in hemolytic transfusion reactions in humans.

Some of the elements involved in a transfusion reaction when A_1 red cells were administered to a sensitized A negative dog are shown in the graphs (Figure 8). Before transfusion the recipient had an anti-A antibody titer of 1/256. The A_1 erythrocytes infused were tagged with Cr^{51} and the rate of *in vivo* hemolysis was determined in the recipient. Five minutes after administration of 20 ml of incompatible cells, no type A_1 cells were detected in a sample of venous blood. Free hemoglobin was detectable in the plasma in 5 minutes and reached a maximum at 20 minutes. These data suggest that the infused incompatible cells were sequestered out of the circulation before they were hemolyzed.[55] Rapid transient thrombocytopenia and leukopenia were also noted. No significant changes in serum antibody or complement titers were observed.

Hemolytic reactions also occur when serum from a dog sensitized to A_1 erythrocytes is administered to a type A dog. Many of the same clinical signs are seen that are present when an A_1 sensitized A negative dog is infused with A_1 erythrocytes. Hemolysis and hemoglobinuria are major features. The severity of the hemolytic reaction is directly proportional to the quantity of antibody administered. Five ml of serum containing a titer of 1/256 anti-A will produce measurable hemolysis in a type A dog. In contrast to the hemolytic reaction resulting from infusion of incompatible cells to a sensitized dog, the period of hemolysis may extend over several days if the dose of antibody is large enough. The recipient's red cells react positively in the antiglobulin test immediately after the antiserum is administered and may remain positive for several weeks.

A hemorrhagic state is frequently encountered as a component of the transfusion reaction in sensitized A negative dogs transfused with A positive blood and in A positive dogs[56] which receive serum containing anti-A-antibodies. Thrombocytopenia with impaired clot formation always accompanies the bleeding state. The thrombocytopenia apparently results not from immune lysis of platelets but rather from a sequestration of platelets out of the active cir-

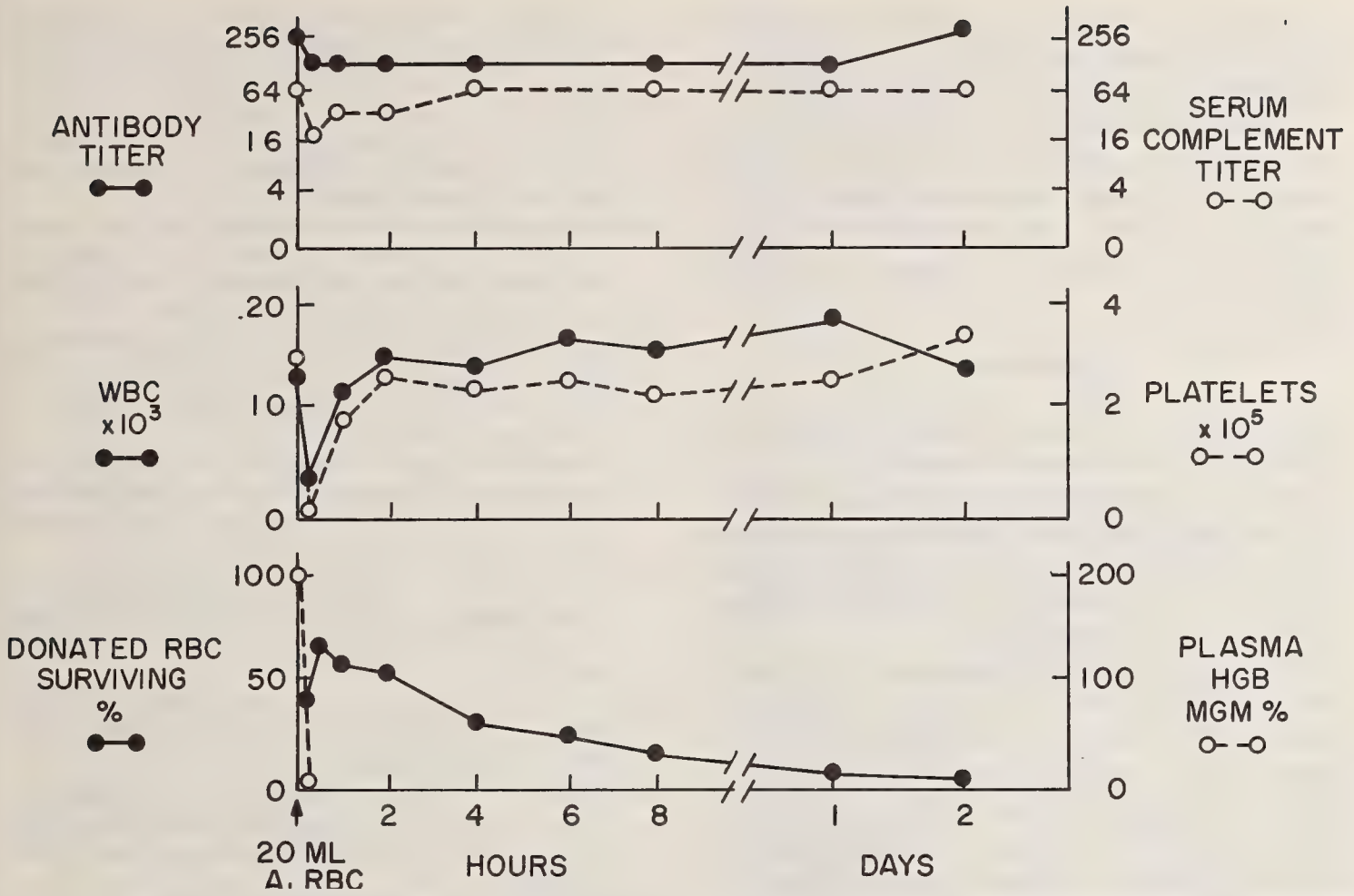


FIGURE 8.—The principal hematologic and serologic changes following infusion of type A_1 red cells to a sensitized A negative dog.[51]

culation, probably as a result of an immune adherence reaction with incompatible erythrocytes in the case of administration of incompatible cells. Platelet sequestration probably results from a similar sequence of events in the case of administration of anti-A-antisera to an A positive dog. In addition to the thrombocytopenia, Friesen, Harsha, and McCroskey[56] have reported fibrinolytic activity and a heparin-like activity in the plasma of recipient dogs resembling the syndrome of disseminated intravascular coagulation.

Clinically significant transfusion reactions involving other canine blood types are rare. Administration of group C blood to a sensitized C negative dog can cause a severe reaction; however, since 98% of dogs are C positive, C incompatibility reactions are of little practical importance. Likewise, F negative dogs are a rarity. B, D, E, and G groups are only weakly antigenic, and clinically recognizable transfusion reactions involving them are infrequent. Hemolytic transfusion reactions have not been described in sheep and those in sensitized cattle run a course similar to those in dogs.

Hemolytic disease of newborn puppies has been seen when type A_1 puppies feed on the clostrum of dams previously sensitized to A_1 erythrocytes by transfusion.[47] Anti A_1- antibodies are adsorbed through the gastrointestinal mucosa into the circulation of the newborn pup resulting in a hemolytic anemia often so severe as to be fatal during the first few days of life. After the first day of life, the gastrointestinal mucosa of the newborn dog apparently loses its ability to absorb active anti-A-antibody, and A positive puppies which have been fed artificially or by a non-sensitized bitch during the first 24 hours may be returned to their dam.

Although in routine veterinary work canine blood is frequently not tested for compatibility before transfusion, in a research laboratory setting where sensitization by prior transfusions may be common, typing should be done to avoid the morbidity which may result from transfusion of incompatible blood and to avoid the mortality seen in hemolytic disease of newborn pups.

SUMMARY

The major erythrocyte antigenic systems of sheep, cattle and dogs are discussed.

The discoveries of the ovine R-O and X-Z systems are reviewed. Major emphasis is placed on a description of the ML system and its relationship to the intracellular potassium concentration of the sheep erythrocyte.

Eleven major cattle blood groups are briefly reviewed. The complexity of the B system is noted, and there is a discussion of the J antigen and its relationship to cells and serum.

The discussion of canine blood groups includes a review of the discovery of the A system and a description of transfusion reactions involving A_1 incompatibility.

REFERENCES

1. Bialosuknia, W., and Kaczkowski, B. Recherches sur les groupes serologiques chez les moutons. Compt. Rend. Soc. Biol. 90: 1196–1198, 1924.
2. Bialosuknia, W., and Kaczkowski, B. On the differentiation of various breeds of sheep by means of serologic methods. J. Immunol. 9:593-601, 1924.
3. Kaczkowski, B. Recherches sur l'heredite des isoagglutinines et des proprietes d'isoagglutination chez les brebis. Compt. Rend. Soc. Biol. 98:386–387, 1928.
4. Kaczkowski, B. Contribution to the studies of the origin of European sheep. Proc. Roy. Soc. Edinburgh 48:10–14, 1928.
5. Andersen, T. Untersuchungen uber die Blutgruppeneigenschaften der Schafe. Zeitschr. Rassenphysiol. 10:88–103, 1938.
6. Stormont, C. An example of a recessive blood group in sheep (Abstract). Genetics 36:577–578, 1951.
7. Rendel, J., Neimann-Sorensen, A., and Irwin, M. R. Evidence for epistatic action of genes for antigenic substances in sheep. Genetics 39:396–408, 1954.
8. Rendel, J. Further studies on some antigenic characters of sheep blood determined by epistatic action of genes. Acta Agr. Scand. 7:224–259, 1950.
9. Amzel, R., Halber, W., and Hirzfeld, L. Vergleichende Untersuchungen uber gruppenspezifische Strukturen verschiedener Tierarten. Zeitschr. Immunitatsforsch. 42:369–408, 1925.
10. Niemann-Sorensen, A., Rendel, J., and Stone, W. H. The J-substance of cattle. II. A comparison of normal antibodies and antigens in sheep, cattle, and man. J. Immunol. 73:407–414, 1954.
11. Rasmusen, B. Blood groups in sheep. I. The X-Z system. Genetics 43:814–821, 1958.
12. Rasmusen, B. Blood groups in sheep. Ann. N.Y. Acad. Sci. 97:306–319, 1962.
13. Ellory, J. C., and Tucker, E. M. Stimulation of the potassium transport system in low potassium type sheep red cells by a specific antigen-antibody reaction. Nature 222:477–478, 1969.
14. Rasmusen, B. A., and Hall, J. G. Association between potassium concentration and serologic type of sheep red blood cells. Science 151:1551–1552, 1966.
15. Rasmusen, B. A. A blood group antibody which reacts exclusively with LK sheep red blood cells. Genetics 61:s49, 1969.
16. Tosteson, D. C., and Hoffman, J. F. Regulation of cell volume by active cation transport in high and low potassium sheep red cells. J. Gen. Physiol. 44:169–194, 1960.
17. Lauf, P. K., Rasmusen, B. A., Hoffman, P. G., Dunham, P. B., Cook, P., Parmelee, M. L., and Tosteson, D. C. Stimulation of active transport in LK sheep red cells by blood group -L-antiserum. J. Membrane Biol. 3:1–13, 1970.
18. Dunham, P. B., and Hoffman, J. B. Correlation of T-ouabain binding and K-transport in HK and LK red cells of sheep. Fed. Proc. 28:339, 1969.
19. Snyder, J. J., Rasmusen, B. A., and Lauf, P. K. The nature of the antibody in iso-immune anti-L sera affecting active potassium transport in LK sheep red cells. J. of Immunol. 107:772–781, 1971.
20. Ferguson, L. C. Heritable antigens in the erythrocytes of cattle. J. Immunol. 40:213–242, 1941.
21. Ferguson, L. C., Stormont, C., and Irwin, M. R. On additional antigens in the erythrocytes of cattle. J. Immunol. 44:147–164, 1942.
22. Stormont, C. Additional gene-controlled antigenic factors in the bovine erythrocyte. Genetics 35:76–94, 1950.
23. Stormont, C. Current status of blood groups in cattle. Ann. N.Y. Acad. Sci. 97:251–268, 1962.
24. Stormont, C. Acquisition of the J substance by the bovine erythrocyte. Proc. Nat. Acad. Sci. 35:227–237, 1949.
25. Stone, W. H., and Irwin, M. R. The J substance of cattle. I. Developmental and immunogenetic studies. J. Immunol. 73:397–406, 1954.

26. PATEL, J. R. Further immunogenetic studies of the J antigenic character of cattle blood. Ph.D. Thesis, Univ. Wis., Madison, Wis., 1958.
27. STONE, W. H. The J substance of cattle. Ann. N.Y. Acad. Sci. 97:269–280, 1962.
28. THIELE, O. W., and KOCH, J. The J blood group substance of cattle. J haptenic activity of glycosphingolipids of serum. Eur. J. Biochem. 14:379–386, 1970.
29. MARCUS, D. M., and CASS, L. E. Glycosphingolipids with Lewis blood group activity: uptake by human erythrocytes. Science 164:553–555, 1969.
30. SPRAGUE, L. M. On the recognition and inheritance of the soluble blood group property "Oc" of cattle. Genetics 43:906–912, 1958.
31. STORMONT, C., MILLER, W. J., and SUZUKI, Y. Blood groups and the taxonomic status of American buffalo and domestic cattle. Evolution 15:196–208, 1961.
32. BRAEND, M. Blood groups of cattle in Norway. Skand. Bladforlag. 144, 1959.
33. NIEMANN-SORENSEN, A. Blood Groups of Cattle: 177. A/S Carl Fr. Mortensen, Copenhagen, Denmark, 1958.
34. RENDEL, J. Studies of cattle blood groups. IV. The frequency of blood group genes in Swedish cattle breeds, with special reference to breed structure. Acta Agr. Scand. 8:191–215, 1958.
35. TOLLE, A. Die Blutgruppen des Rindes: 196 Verlag M. and H. Schaper, Hanover, Germany, 1960.
36. COHEN, C., and FULLER, J. L. The inheritance of blood types in the dog. J. Heredity 44:225–228, 1953.
37. VON DUNGERN, E., and HIRZFELD, L. Uever nachweis und venerbung biochemischer strukturen. Z. Immunitatsforsch. 4:531–540, 1910.
38. OTTENBERG, R., KALISKI, D. J., and FRIEDMAN, S. S. Experimental agglutinative and hemolytic transfusions. J. Med. Research 28:141–163, 1913.
39. MCENERY, E. T., IVY, A. C., and PECHOUS, C. E. On the presence of iso-agglutinins in the blood of dogs. Am. J. Physiol. 68:133–141, 1924.
40. MELNICK, D., BURACK, E., and COWGILL, G. R. Development of incompatibilities in dogs by repeated infusion of red blood cells. Proc. Soc. Exptl. Biol. Med. 33:616–620, 1935.
41. HAHN, P. F., and BALE, W. F. Linear relationship between the circulating red cell mass and the venous hematocrit as determined with radioactive iron. Am. J. Physiol. 136:314–317, 1942.
42. HOLMAN, R. L., MAHONEY, E. B., and WHIPPLE, G. H. Blood plasma protein regeneration controlled by diet. I. Liver and casein as potent diet factors. J. Exptl. Med. 59:251–267, 1934.
43. WRIGHT, A. Isochemolysins and isoagglutinins occurring in dogs. Proc. Soc. Exptl. Biol. Med. 34:440–443, 1936.
44. CHRISTIAN, R. M., ERWIN, D. M., and YOUNG, L. E. Observations on the in vitro behavior of dog isoantibodies. J. Immunol. 66:37–50, 1951.
45. CHRISTIAN, R. M., STEWART, W. B., YUILE, C. L., ERVEIN, D. M., and YOUNG, L. E. Limitation of hemolysis in experimental transfusion reactions related to depletion of complement and isoantibody in the recipient. Blood 6:142–150, 1951.
46. SWISHER, S. N., IZZO, M. J., and YOUNG, L. E. A method of quantitative differential agglutination of dog erythrocytes. J. Lab. Clin. Med. 41:936–945, 1953.
47. SWISHER, S. N., IZZO, M. J. and YOUNG, L. E. Post transfusion survival of fresh and stored dog erythrocytes measured by differential agglutination. J. Lab. Clin. Med. 41:946–952, 1953.
48. YOUNG, L. E., ERVIN, D. M., and YUILE, C. L. Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma. I. Serologic and hematologic aspects. Blood 4:1218–1231.
49. YOUNG, L. E., CHRISTIAN, R. M., ERVIN, D. M., DAVIS, R. W., O'BRIEN, W. A., SWISHER, S. N., and YUILE, C. L. Hemolytic disease in newborn dogs. Blood 6:291–313, 1961.
50. YOUNG, L. E., O'BRIEN, W. A., MILLER, G., SWISHER, S. N., ERWIN, D. M., CHRISTIAN, R. M., and YUILE, C. L. Erythrocyte-isoantibody reactions in dogs. Trans. N.Y. Acad. Sci. Ser. II 13:209–213, 1951.
51. SWISHER, S. N., and YOUNG, L. E. The blood grouping systems of dogs. Physiologic Reviews 41:495–520, 1961.
52. STOHLMAN, F. Enhancement of canine isohemagglutinin A by a heat labile serum factor, probably complement. Proc. Soc. Exptl. Bio. Med. 82:562–566, 1953.
53. DUDOK, C., COENEGRACHT, N., POLL, P., and LINDE, J. The practical importance of blood groups in dogs. J. Small Anim. Pract. 8:285–289, 1967.
54. YUILE, C. L., VANZANDT, T. F., ERWIN, D. M., and YOUNG, L. E. Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma. II. Renal aspects following whole blood transfusions. Blood 4:1232–1239, 1949.
55. SWISHER, S. N. Studies of mechanisms of erythrocyte destruction initiated by antibodies. Trans. Assoc. Am. Physicians 67:124–132, 1954.
56. FRIESEN, S. R., HARSHA, W. N., and MCCROSKEY, C. H. Massive generalized wound bleeding during operation with clinical and experimental evidence of blood transfusion reactions. Surgery 32:620–629, 1952.

DISCUSSION

CHAIRMAN SEEGERS: May we assume, Dr. Stengle, that this review represents a thorough search of the literature? Do any of you wish to ask some questions or make comments? Any of you, please.

J. MOOR-JANKOWSKI, New York University School of Medicine, New York: I have a general question to ask Dr. Stengle. We have been evaluating in New York the importance of our own group in blood groups of nonhuman primates. It would help us to have an idea how important in scientific research are the blood groups of dogs, cattle, and sheep. We know of course that cattle and sheep are used for commercial evaluation, stock-breeding, and so on. Dogs are used most probably for research. But you probably have an overview of this and maybe there are other people here who would like to know how far information which you presented now is being used in scientific research.

DR. STENGLE: I think there are probably several possible answers to it, Dr. Moor-Jankowski. I think science justifies itself, really, and so that's one level of answer; but you're asking about perhaps a more practical level. To how many people in the scientific communiy is it important to know all there is to know about dogs, sheep, cattle, and primates blood grouping and the problems that will arise as they do cross-transplantations or transfusions among these species? I don't have a ready answer for that. I think it's an area of science which needs to be continued. I can't give you any figures.

C. J. STORMONT, University of California, Davis, Calif.: I'd like to comment, Dr. Stengle. The whole area of animal blood group research, especially the larger animals, cattle, sheep, horses, goats, pigs, is very poorly supported in the United States. In fact, we are being literally squeezed out of business by lack of grant funds. I can substantiate this in more ways than one. Some labs are fortunate; they seem to get continuing NIH support. New labs try to obtain support, but they can't because the money is all going to one lab. This is really the truth. I don't know where we're going to go for our money for research on blood groups in large animals. It looks to me like we're going to have to get our support directly from industry, because the U.S. Department of Agriculture doesn't seem to have any money, at least to support people in *this* country. They do support European investigators. They bring them over here to visit our labs even though they never seem interested in supporting us. I could go on and on about this thing and really blast.

So, as I say, it looks as if we're going to have to get support directly from industry. You'll have to pay for it when you buy beef. It'll cost you ½ cent more a pound, or something like this. My only support now comes from providing services—typing work. I do not get one penny of grant money for research on animal blood groups. The horse blood typing is now being supported by five horse breeder organizations, simply because of the importance of the blood typing tests in resolving questions of parentage arising in the registration of pure-bred horses. This is the main support at the moment.

In the sheep industry, blood typing costs more than some of the larger animals involved in the parentage cases, so they don't utilize it in solving parentage cases. With cattle, they do.

W. J. DODDS, New York State Department of Health, Albany, N.Y.: I want to make a practical comment about blood groups in dogs. I'm a firm believer that the animal blood groups should be studied more from a clinical point of view. We have a colony of dogs with hereditary bleeding defects, and we have to transfuse them frequently. Because we have all the typings here for dogs, we supply this service free to the veterinary industry in the Capitol area. We have now been able to type the regular donors. We type all the blood bags before they are used so they know which blood to use for transfusion purposes. We can only do this because our research is funded from NIH for another source.

CHAIRMAN: I might call your attention, Dr. Stormont, to a nice article in the last issue of *Chemists,* a publication of the Institute of Chemists, on what kinds of funds ought to be made available for research. He makes the point that it's silly to ask for a million dollars. Until scientists enter the class of big spenders, it's no use yelling. The author points out why, and so on.

DR. STORMONT: I'd like to make one other comment here. I enjoyed your remarks about the high and low potassium being associated with the M system of sheep blood groups. This is a really remarkable observation. In fact, Arasmus and my former student, Ann King, working in Edinborough, made the connection be-

tween the M system of sheep and high and low potassium levels. Then, Tucker and Alleray at Babram in Cambridge made the connection between the relationship of the antibody on the membrane and altering the high and low potassium types. Physiologists are crazy about stuff like this. They write to me and ask if I have any anti-L. I have to write back that I'm sorry we ran out of sheep blood typing simply because we had lack of support. I add that if they can get us any support we'd get back in and make some good anti-L, but I can't do it without support.

FACTORS REGULATING THE PRODUCTION OF COAGULATION ACTIVITIES IN PERFUSED ORGANS

W. J. Dodds* and L. W. Hoyer**

Isolated rabbit livers and spleens were perfused first for 1 hr to release stored coagulation activity and then for another 4 hr as previously described.[1] The perfusate (80 ml) was devoid of coagulation activity and consisted of washed rabbit blood cells, human albumin in Tyrode's solution and trisodium citrate. At 30 min of the second perfusion period, 5 ml of plasma from normal individuals or from those with congenital or acquired defects of coagulation were added to perfusates. Subsequent production of coagulation factors was inversely proportional to perfusate coagulation activity, an observation which suggests regulation by a negative feedback process. The effect of splenic perfusion with factor VIII deficient plasma from patients with CRM (−) and (+) hemophilia A and von Willebrand's disease was compared; plasma was designated as CRM (+) if it contained material which cross-reacted with human anti-factor VIII. As would be expected for a system regulated by negative feedback, factor VIII production by the spleen continued while factor IX production ceased. In addition, a marked enhancement of splenic factor VIII activity was observed in the presence of CRM(+) hemophilia A plasma but not with CRM(−) or von Willebrand's plasmas ($p = < 0.01$). Experiments with factor IX deficient plasma from patients with CRM(−) and (+) hemophilia B demonstrated a similar but less striking enhancement of factor IX activity in the presence of CRM(+) hemophilia B plasma as compared with CRM(−) plasma ($p = < 0.04$). CRM(+) plasma contained material which cross-reacted with human anti-factor IX. These observations suggested that antigenically detectable but nonfunctional molecules accumulate in CRM(+) plasmas and can be converted rapidly by the spleen to active coagulation factors. Alternatively, inactive precursors of factor VIII and IX in CRM(+) plasma could stimulate splenic factor VIII and IX production, respectively.

INTRODUCTION

The liver is known to play a major role in the synthesis of plasma proteins, including the coagulation factors, fibrinogen, prothrombin, and factors V, VII, IX, and X.[2] Recent studies, however, have implicated the liver, spleen and kidney as sites of synthesis and/or storage of factor VIII.[1-3] Other sites for factor VII and IX synthesis have also been proposed.[1,2] In previous organ perfusion experiments, the appearance of coagulation factor VII, VIII, and IX activities gradually increased with time and was prevented by inhibitors of protein synthesis.[1] The experimental approach was different from that of other investigators because an initial hour of perfusion was included to permit release into the perfusate of tissue-stored coagulation factors.[1] This was followed by a second perfusion period of 4 hr with a fresh aliquot of perfusate. The current studies were designed to investigate the possibility that a negative feedback process regulated the appearance of coagulation activities during both the initial hour of perfusion (release period) and the final 4 hr of perfusion (synthesis period).

MATERIALS AND METHODS

Rabbit livers and spleens were isolated, prepared, and perfused as described previously.[1,3] The perfusate was devoid of coagulation activity and consisted of 80 ml of the following mixture: 25 ml of washed rabbit blood cells, 47 ml of 6% human albumin in Tyrode's solution, and 8 ml of 3.8% trisodium citrate. Final citrate concentration was maintained at 0.38% by addition of 1–2 ml of 3.8% citrate hourly; without this additive platelet-fibrinous masses formed and became trapped in the perfusion filter. Initial experiments with heparin were discontinued because heparinized perfusates produced an increased peripheral vascular resistance and arterial perfusion pressure, and a tendency to-

* Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201.

** Department of Medicine, University of Connecticut School of Medicine, Hartford, Connecticut 06105.

*** Supported in part by U.S. Public Health Service Grant HL-09902 and by a Veterans Administration research grant.

wards fragmentation of circulating platelets; these heparin-induced changes were particularly noticeable in kidney perfusions. Platelet counts in the citrated perfusion system adopted subsequently, remained relatively constant at 40,000–100,000/cu mm throughout the perfusion period. As described above, an initial hour of perfusion, the "flush" period, was included and followed by a second perfusion period of 4 hr with a fresh aliquot of perfusate.[1] Perfusate was circulated through the organs at a rate of 7–10 ml/min; greater flow rates were associated with platelet damage, hemolysis, and nonspecific activation of coagulation factors. With this technique, perfused organs maintained their physiologic function; livers incorporated radio-labeled leucine into albumin and kidneys sustained the uptake of radioactive vitamin B_{12} bound to transcobalamin II.[4] Production of coagulation factor VII, VIII, and IX activities in livers and spleens increased with time and was sensitive to puromycin, cycloheximide, and actinomycin D.[1-3] Subsequent investigation of these factor VIII and IX activities demonstrated properties similar to plasma coagulation factors VIII and IX as well as the capacity to neutralize human anti-factor VIII and anti-factor IX, respectively.[2,5] Perfused kidneys also generated factor VII, VIII, and IX activities but, of these, only factor VII was sensitive to inhibitors of protein synthesis.[1] In addition, kidney factor VIII activity was not inactivated by human anti-factor VIII.[2,5]

In the present studies, 5 ml of citrated plasma from normal individuals or from those with congenital or acquired defects of coagulation were added to hepatic and splenic perfusates at 30 min of the second perfusion period. The plasmas used were either fresh or fresh-frozen and of the following types: normal rabbit or human; warfarin-treated rabbit; factor VIII and IX deficient with and without demonstrable cross-reacting material (CRM); factor VIII deficient from patients with von Willebrand's disease (VWD). CRM(−) and (+) hemophilia A plasmas were obtained from patients previously reported by one of us.[6,7] CRM(−) and (+) hemophilia B plasmas were kindly supplied by Dr. H. R. Roberts of Chapel Hill, N. C.[8] Von Willebrand's disease plasmas were generously supplied by Dr. E. J. W. Bowie of Rochester, Minn.[9] Normal rabbit and human plasmas had between 80 and 160% of the coagulation activity found in a pooled standard plasma obtained from rabbits and humans, respectively. Paired experiments were performed with fresh normal rabbit plasma added to perfusates, and then with plasma from the same rabbit after pretreatment with 2.5 mg/kg sodium warfarin (Endo Laboratories, Inc., Garden City, N. Y.) twice daily for 3–5 days. The prothrombin time of this plasma was > 2 min and factor VII and IX activities were reduced to < 3% of normal; factor VIII activity varied from 48 to 200% with an average of 124%. Normal human plasma used in perfusions was obtained from 4 healthy volunteers who had not taken any medication for at least 7 days. Each plasma was divided into equal 4–5 ml aliquots; one aliquot was used immediately and the others frozen and stored at −70° C for subsequent experiments. All deficient plasmas were fresh-frozen, stored at −70° C until used, and were compared in assays to a pooled standard human plasma. CRM(−) factor VIII deficient plasma had < 1% factor VIII activity, but normal levels of the other coagulation factors. CRM(+) factor VIII deficient plasma had 3–9% factor VIII with normal factor IX activity. CRM(−) and (+) factor IX deficient plasmas had < 1% and 7–9% factor IX activity, respectively, with low normal (45–78%) factor VIII activity. Von Willebrand's disease plasma had 2–8% factor VIII activity with normal factor IX activity. In some experiments, factor VIII concentrates, prepared by cryoprecipitation of normal human plasma, were dissolved in the 5-ml aliquot of normal or deficient plasma added to splenic perfusates. This was accomplished by centrifugation of the cryoprecipitate, careful removal of all supernatant plasma, and dissolving the precipitate in the appropriate sample.

Perfusate samples (3 ml) were taken at the end of the initial hour of perfusion, termed "flush," and at zero, ½, ¾, 1, 1½, 2, 3, and 4 hr of the final perfusion period. Perfusion volume was maintained after sampling by addition of 3 ml of fresh perfusate. All samples were prepared and assayed for factor VIII and IX activities with canine deficient plasmas in a one-

stage assay as previously described.[1] Some samples were also assayed with human deficient substrate in a similar one-stage assay.[10] There was good agreement between the activities measured in perfusates by these two methods. However, because the addition of human plasma was studied in a rabbit perfusion system, the relative coagulation activities of rabbit and human factors VIII and IX were also determined.

Coagulation activity of perfusates as shown in Figures 1–6 of "Results" refers to the percent activity compared to that of a *standard pool of normal rabbit plasma which was assigned a value of 100%*. All coagulation data were computed and the best fit plot was obtained after fifth order regression analysis; the standard deviation at each sample interval was also determined.

RESULTS

Figure 1 illustrates the control data for factor VIII and IX activities produced by livers and speens perfused without additives. The activity present at the end of the initial hour of perfusion, labeled "flush," has been attributed primarily to release of tissue stores.[1] Factor VIII activity (Figure 1a) is more active during splenic perfusion although perfused livers sustained their ability to increase perfusate factor VIII activity throughout the 4 hr perfusion period. In contrast to factor VIII, there was no difference between splenic and hepatic factor IX production (Fig. 1b). The activities generated in the experiments shown in Figure 1 were efficiently blocked by puromycin, cycloheximide, and actinomycin D.[1-3] The data shown in subsequent figures include only the perfusion period following addition of plasma (the interval enclosed within the stippled areas on Figure 1).

Data for splenic and hepatic factor VIII and IX activities after addition of fresh normal rabbit plasma are shown in Figure 2. Perfusate factor VIII and IX activities were increased after addition of normal plasma and both factor VIII (Figure 2a) and factor IX (Figure 2b) production ceased. As might be expected by the

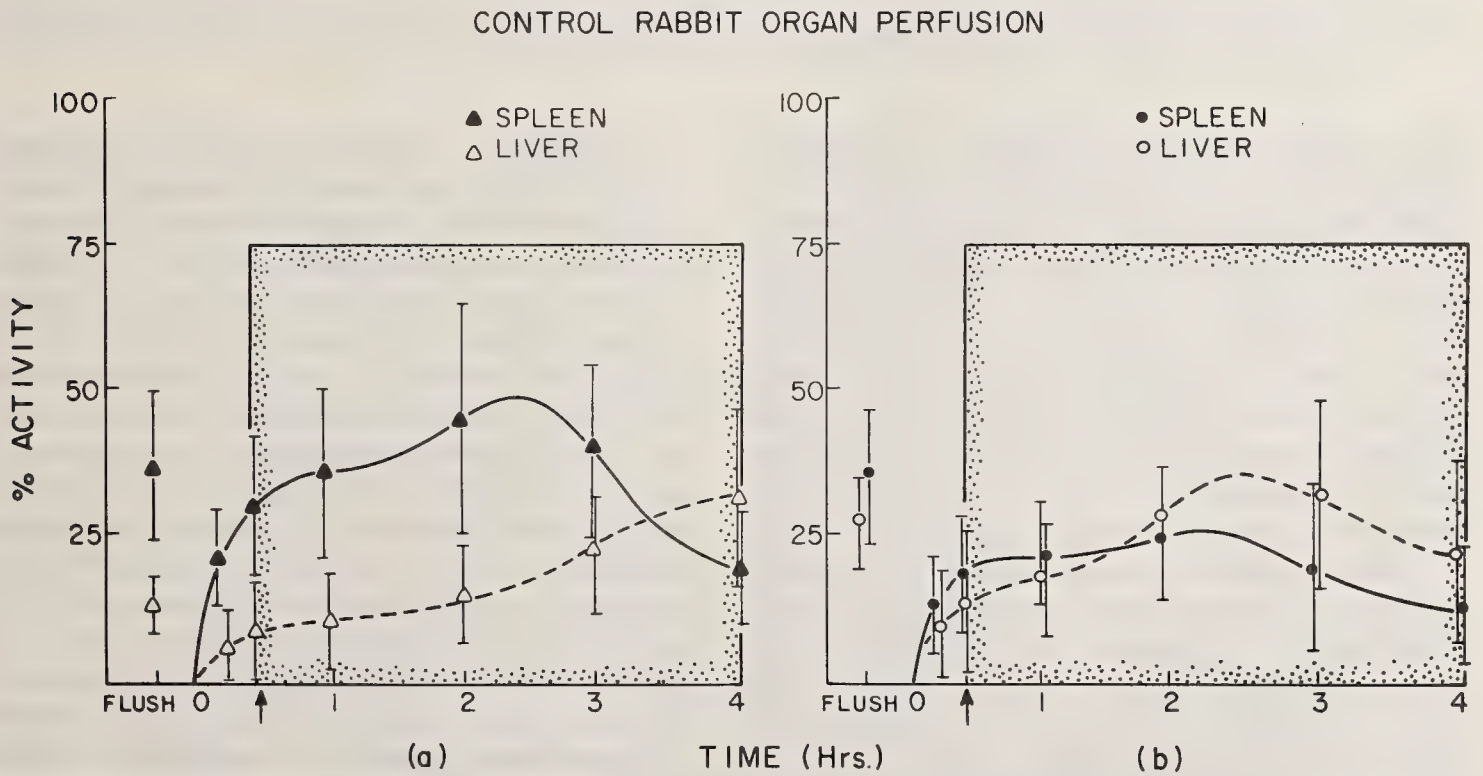


FIGURE 1.—a. Factor VIII activities of 20 control rabbit spleen and 24 control rabbit liver perfusions. b. Factor IX activities of the same experiments shown in 1a. Areas enclosed by stippling represent the period of perfusion shown on subsequent figures. "Flush" values are activities after the initial hour of perfusion. Data computed and best fit curve obtained by fifth order regression analysis; limits shown represent the standard deviation at each sample point.

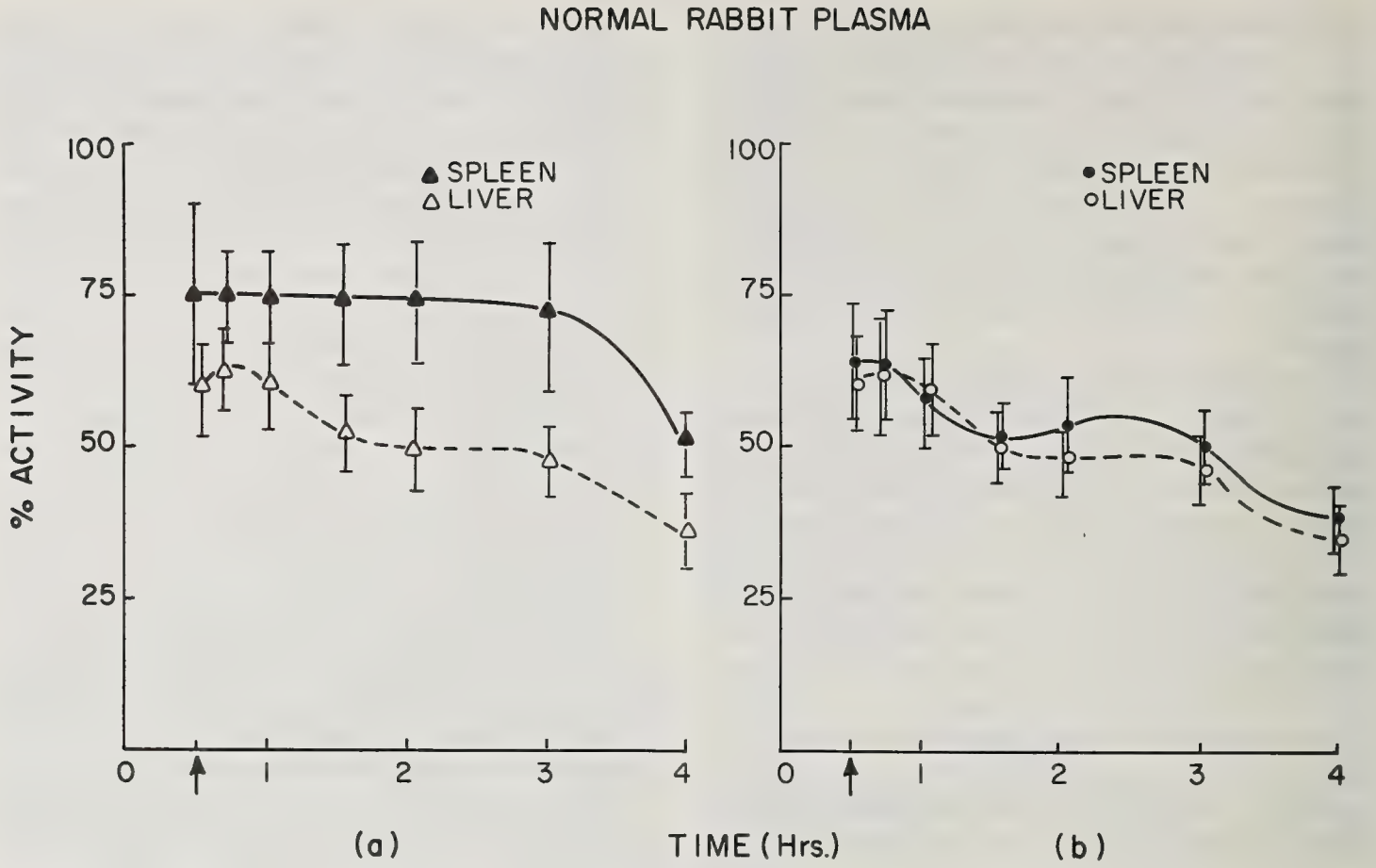


FIGURE 2.—a. Factor VIII activities of 4 spleen and liver perfusions after addition of 5 ml fresh normal rabbit plasma as indicated by the arrow. b. Factor IX activities of the same experiments shown in 2a.

greater factor VIII activity at 30 min in control splenic perfusions as compared with hepatic perfusions (Figure 1a), the addition of normal plasma to splenic perfusates (Figure 2a) resulted in slightly higher factor VIII activity in the spleen than in the liver ($p = < 0.05$). In addition, factor IX activities immediately after addition of normal plasma were equivalent in perfusates from both organs, a result which would be predicted on the basis of similar control levels of factor IX at 30 min (Figure 1b).

Figure 3 presents data following addition of plasma from the rabbits used in the experiments shown in Figure 2, but after these same animals had been treated with warfarin. The plasma obtained from these warfarin-treated rabbits was factor VII and IX deficient. It can be seen that factor VIII production ceased in perfused livers and spleens (Figure 3a) while factor IX production continued (Figure 3b). Hepatic factor IX production in these experiments appeared similar to that observed in control perfusions without additives (Figure 1b).

Studies following addition of normal human plasma are shown in Figure 4. Perfusate coagulation activities were increased after addition of normal plasma, but it can be seen in Figure 4a that neither fresh plasma nor fresh-frozen plasma was capable of raising the perfusate factor VIII activities sufficiently to inhibit further production. These findings are not surprising if one considers the relatively high factor VIII levels of most animal species as compared with those in man. It was determined in six paired experiments that rabbit plasma was 2.5 (range 1.9 to 3.0) times more active with respect to factor VIII activity but only 1.1 (range 0.9 to 1.5) times more active in factor IX activity than human plasma. Fresh-frozen normal human plasma contained relatively less factor VIII (50–75%) than the same samples that had not been frozen. The generation of splenic factor VIII activity with frozen plasma was more rapid between 30 min and 3 hr than that ob-

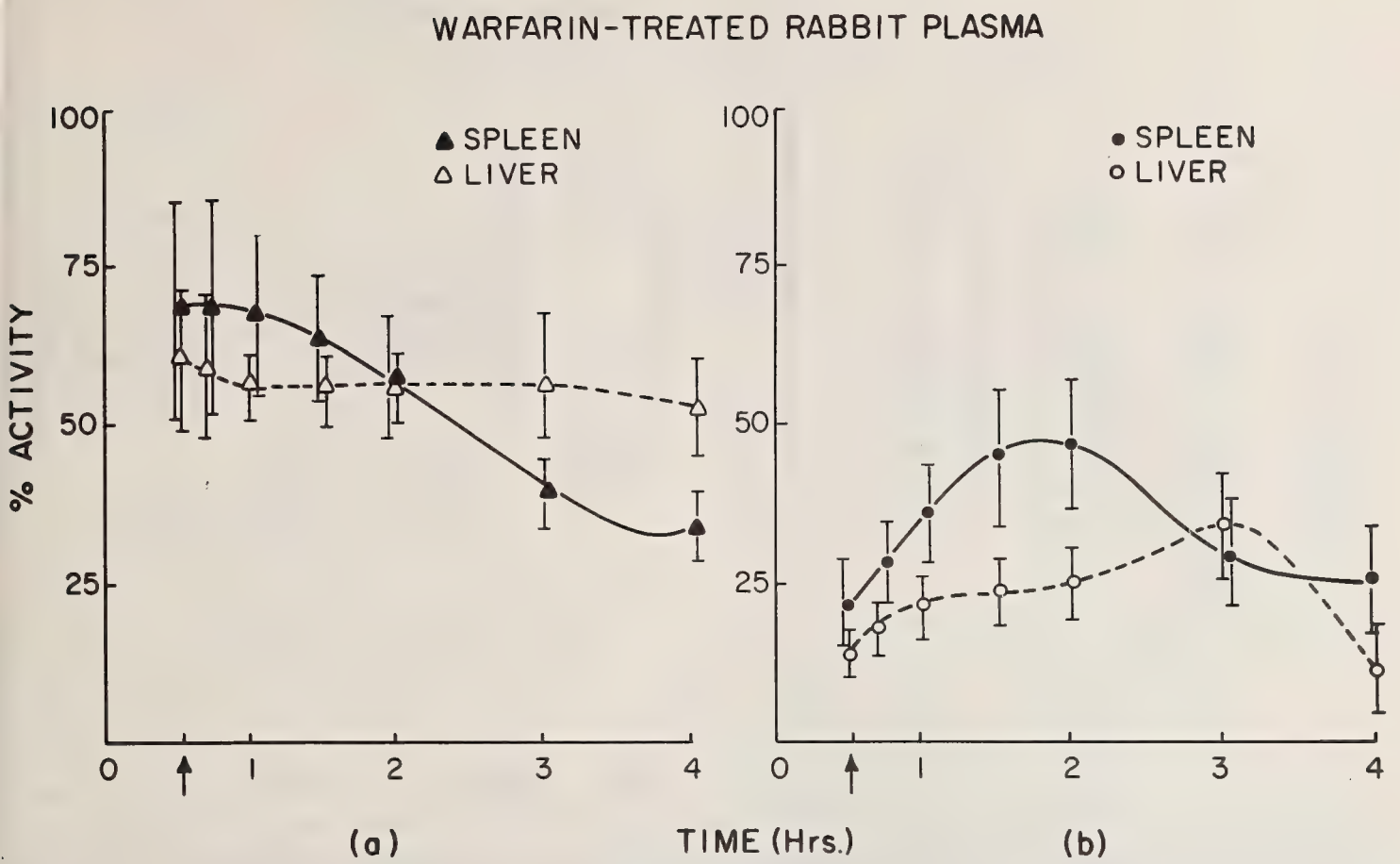


FIGURE 3.—a. Factor VIII activities of 4 spleen and liver perfusions after addition of 5 ml fresh plasma from warfarin-treated rabbits as indicated by the arrow. b. Factor IX activities of the same experiments shown in 3a.

served with fresh plasma ($p = < 0.02$), indicating a possible inverse relationship between initial perfusate activity and subsequent production. Similarly, this concept of negative feedback is supported by the data obtained following addition of factor VIII concentrates to normal plasma (Figure 4a). Factor VIII levels, in this instance, were sufficiently elevated to minimize any further increases. Figure 4b shows splenic factor IX activity during the same experiments with normal human plasma. In contrast to the data with regard to factor VIII, the relatively low levels of perfusate factor IX activity achieved were high enough to inhibit further production by the spleen. These findings were consistent despite the use of fresh, fresh-frozen, or frozen plasma with added factor VIII concentrate (Figure 4b). The data are plausible if one considers that little difference exists between the relative activities of human and rabbit factor IX.

Figure 5 presents data obtained after addition of factor VIII deficient human plasma to rabbit spleen perfusates. As would be expected by a system regulated by negative feedback, factor VIII production continued (Figure 5a) while factor IX production ceased (Figure 5b). The factor VIII activity produced with CRM(−) factor VIII deficient and VWD plasmas was similar to that observed in control perfusions (Figure 1a). However, an unexpected finding was the significantly greater effect on splenic factor VIII production after addition of CRM(+) factor VIII deficient plasma. The differences shown in Figure 5a between CRM(−) and (+) plasmas and between CRM(+) and VWD plasmas are statistically significant with $p = < 0.01$. As shown in Figure 5b, there was a tendency for factor IX activity to increase somewhat after addition of VWD plasma in contrast to plasmas from either CRM(−) or (+) classic hemophiliacs. These findings are not understood, but might be related to the ex-

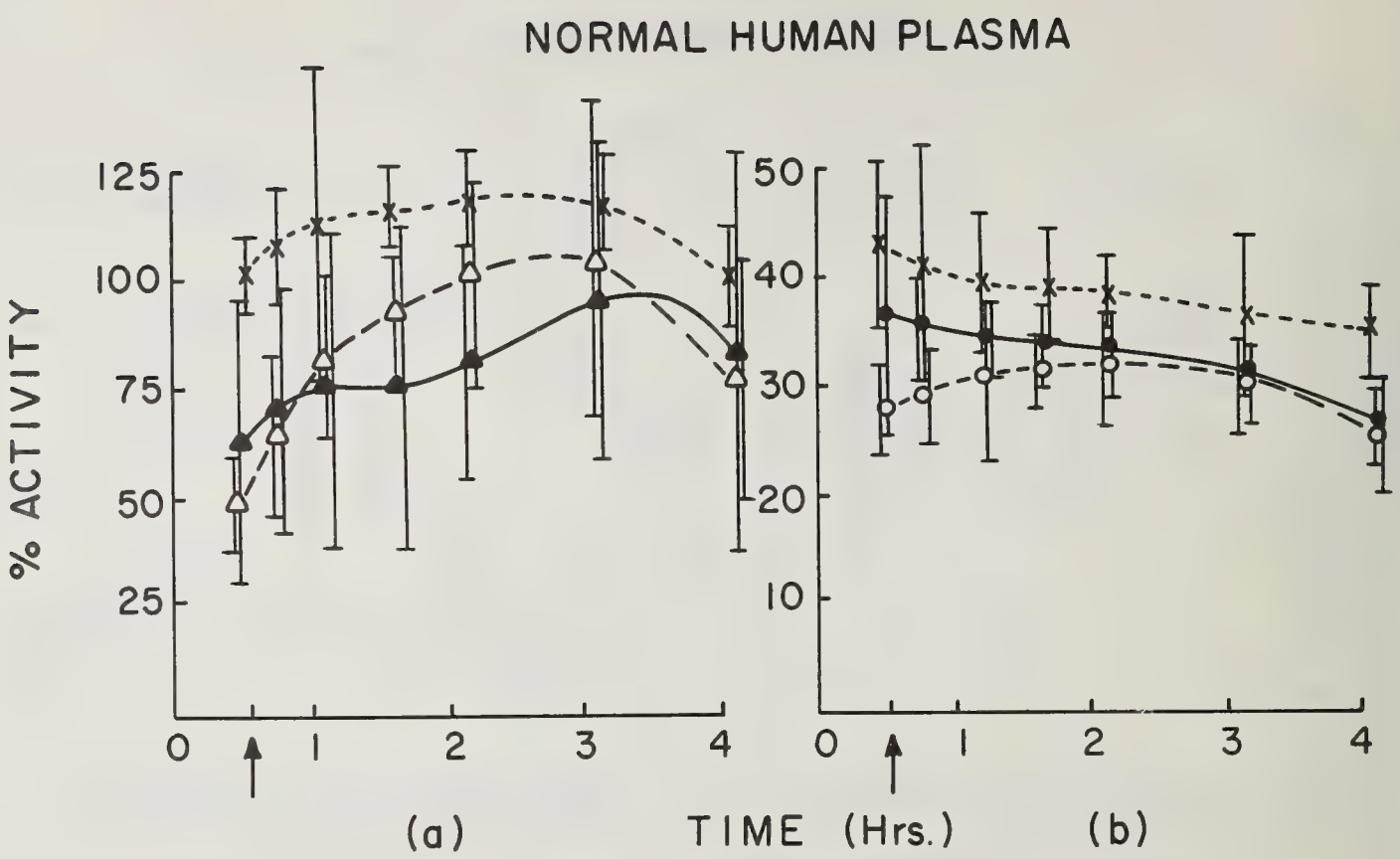


FIGURE 4.—a. Splenic factor VIII activities after addition of 5 ml normal human plasma as indicated by the arrow. Activity of 4 perfusions in each group. Fresh plasma (▲——▲); fresh-frozen plasma (△- - -△); fresh-frozen plasma plus factor VIII concentrate (X·····X). b. Splenic factor IX activities of the same experiments shown in 4a. Fresh plasma (●——●); fresh-frozen plasma (○- - -○); fresh-frozen plasma plus factor VIII concentrate (X·····X).

istence of low normal factor IX activity in some patients and families with VWD.[11]

Figure 6 shows experiments with factor IX deficient human plasma. Interpretation of the data obtained with CRM(−) and (+) factor IX deficient plasma, however, is complicated by the low levels (45 and 78%, respectively) of factor VIII activity present in these frozen plasma samples. The consequence of this low activity was to maintain splenic perfusate factor VIII activity at a level which permitted subsequent increases in factor VIII production (Figure 6a). As would be predicted, CRM (−) hemophilia B plasma with 45% factor VIII activity produced a more sustained increase in splenic factor VIII activity than CRM (+) hemophilia B plasma with 78% factor VIII activity. This observed increase with CRM (−) plasma could be completely prevented by prior mixing of factor VIII concentrate with the CRM (−) factor VIII deficient samples (Figure 6a). Figure 6b demonstrates splenic factor IX production during these experiments. Data obtained with CRM(−) hemophilia B plasma were similar to those with control perfusions (Figure 1b). There was, however, slightly more factor IX activity produced with CRM(+) hemophilia B plasma as compared with CRM(−) plasma ($p = < 0.04$). The change in the pattern of activity resulting from addition of factor VIII concentrate to CRM(−) factor IX deficient plasma (Figure 6b) is not understood.

DISCUSSION

Factor VIII and IX coagulation activities appear in liver and spleen perfusates when these organs are perfused with fluid devoid of coagulation activity (Figure 1). The activities generated in this system have properties similar to plasma coagulation factors VIII and IX,[2,5] and

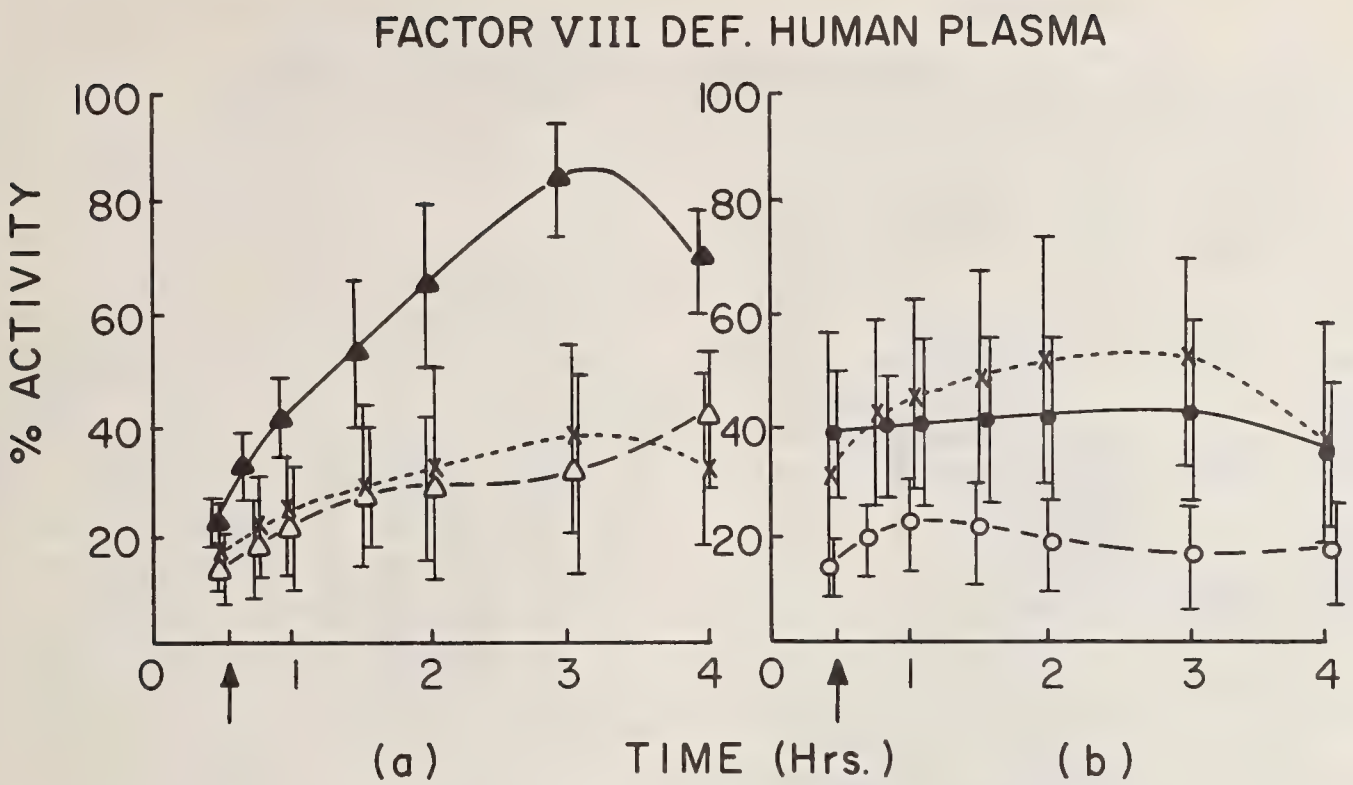


FIGURE 5.—a. Splenic factor VIII activities after addition of 5 ml fresh-frozen factor VIII deficient human plasma as indicated by the arrow. Activity of 4 perfusions in each group. CRM(+) hemophilia A plasma (▲——▲); CRM(−) hemophilia A plasma (△---△); von Willebrand's disease plasma (X·····X). b. Splenic factor IX activities of the same perfusion experiments shown in 5a. CRM(+) hemophilia A plasma (●——●); CRM(−) hemophilia A plasma (○---○); von Willebrand's disease plasma (X·····X).

are sensitive to puromycin, cycloheximide, and actinomycin D.[1-3] The results clearly indicate that perfusion with plasma is not necessary in this experimental model. Small amounts of plasma were incorporated into the system, however, in order to demonstrate that production of activity could be regulated by negative feedback.

Data shown in Figures 2–6 and the results obtained in previous experiments with deficient canine plasmas[5] support the concept that factor VIII and IX production in perfused organs is inversely proportional to the level of these coagulation factors in the perfusate. For example, both normal rabbit and human plasma were capable of elevating perfusate coagulation activities to a level which inhibited further production (Figures 2 and 4). In the case of normal human plasma, however, it required addition of factor VIII concentrates to achieve this effect (Figure 4). These results were anticipated because human plasma is 2.5 times less active than rabbit plasma with respect to factor VIII activity. Similar results in support of the concept of negative feedback were obtained previously with normal and factor VII deficient canine plasmas which both contained normal levels of factors VIII and IX.[5] A reciprocal response between factor VIII and IX production was observed when plasma from warfarin-treated rabbits (factor IX deficient) and from patients with hemophilia A, hemophilia B, and von Willebrand's disease were perfused (Figures 3, 5, 6). Factor IX activity increased in the absence of significant change in factor VIII activity when factor IX deficient plasmas were perfused, whereas the reverse occurred when factor VIII deficient plasmas were used. Factor VIII perfusate data obtained with human factor IX deficient plasma (Figure 6a) were complicated by the relatively low factor VIII activity in these plasma samples. It was possible, however, to reverse this effect on factor VIII production by prior addition of factor VIII concentrates to the hemophilia B samples (Figure 6a). Experiments reported previously following

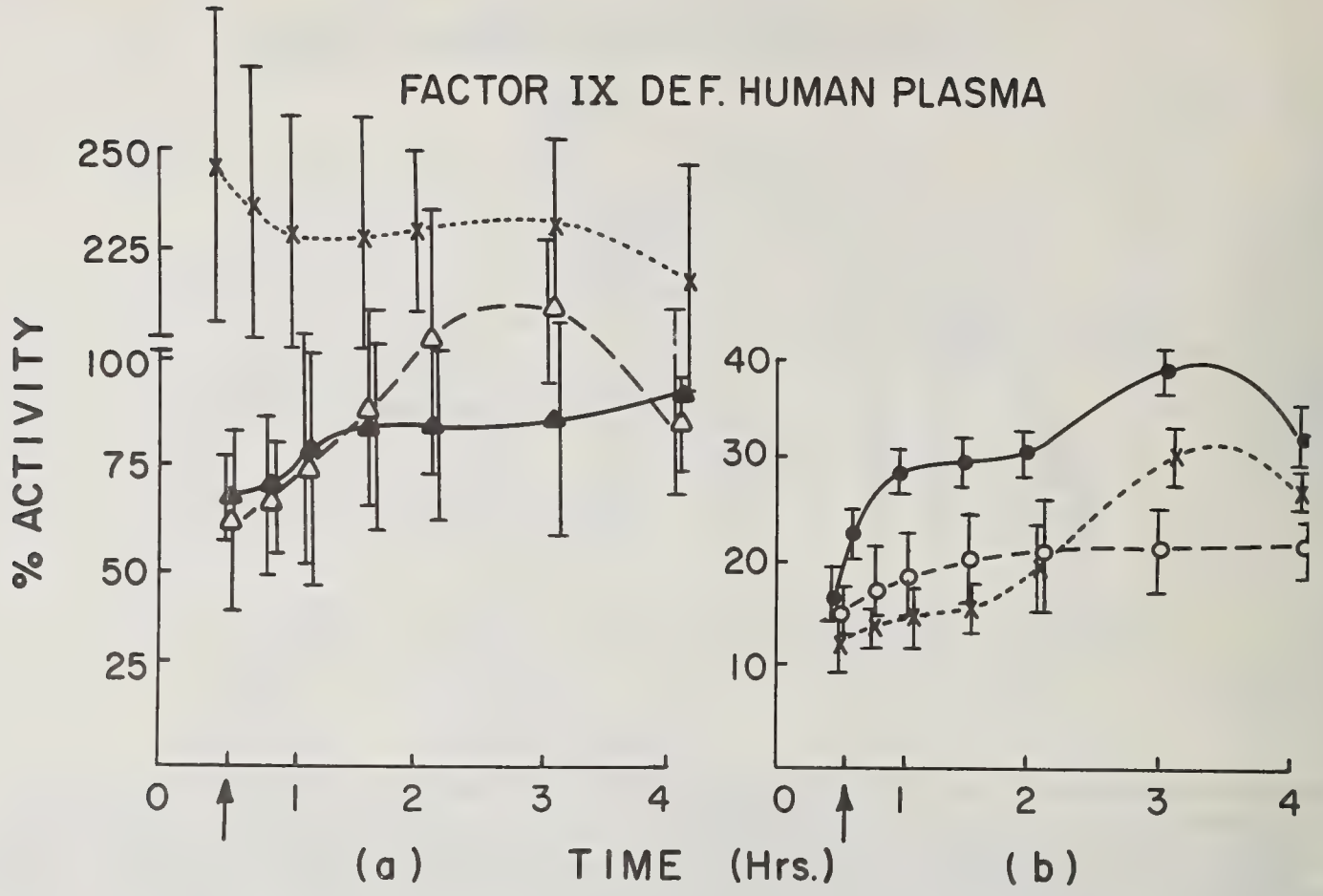


FIGURE 6.—a. Splenic factor VIII activities after addition of 5 ml factor IX deficient human plasma as indicated by the arrow. Activity of 2 perfusions with CRM(+) hemophilia B plasma (▲——▲); 4 perfusions with CRM(−) hemophilia B plasma (△- - -△); 2 perfusions with CRM(−) hemophilia B plasma plus factor VIII concentrate (X X). b. Splenic factor IX activities of the same perfusion experiments shown in 6a. CRM(+) hemophilia B plasma (●——●); CRM(−) hemophilia B plasma (○- - -○); CRM (−) hemophilia B plasma plus factor VIII concentrate (X·····X).

addition of canine factor VIII and IX deficient plasmas demonstrated a parallel inverse relationship between the level of coagulation activity in perfusates and the subsequent production of coagulation factors by the organs perfused.[5] It is interesting that in the current experiments, plasma from animals with an artificially induced coagulation defect (Figure 3) behaved in a manner similar to that obtained from humans (Figure 6) or dogs[5] with an inherited defect.

Although data obtained with deficient human plasmas were consistent with the negative feedback hypothesis proposed from studies with animal plasmas,[5] there was an additional more interesting finding. The splenic response to plasma from patients with CRM(+) hemophilia A was significantly greater than that seen with CRM (−) hemophilia A or VWD plasmas (Figure 5). A similar but less striking enhancement was observed with CRM(+) hemophilia B plasma as compared with CRM(−) hemophilia B plasma (Figure 6). These results suggested that antigenically detectable but nonfunctional molecules accumulate in plasma with CRM and can be converted rapidly by the spleen to active coagulation factors. Alternatively, inactive precursors in plasma with CRM could stimulate splenic release and/or synthesis of coagulation activity.

It has recently been reported, however, that factor VIII antigen is detectable in plasma from all patients with hemophilia A but not from those with VWD.[12-14] These studies employed rabbit antibodies to human factor VIII in contrast to previous experiments with spon-

taneous anti-factor VIII anticoagulants.[6,7] Moreover, new data have shown that the different findings with human and rabbit antibodies to factor VIII are related to differences in antigenic determinants on the molecule with which they react, as well as to the divergent properties of these two kinds of antibodies.[12] The data reported in Figures 5 and 6 of the current studies reveal that the rabbit spleen is capable of discriminating between plasmas obtained from CRM(−) and (+) hemophiliacs in a manner similar to the human antibodies to factors VIII and IX.[6-8] In addition, there was no difference between the activity produced after addition of CRM(−) and VWD plasmas to the splenic perfusion system. There are several possible explanations for the enhanced production of factors VIII and IX in spleens perfused with CRM (+) plasma but these observations require further study.

SUMMARY

Production of coagulation factor VIII and IX activities by isolated perfused livers and spleens was inversely proportional to the coagulation activities of the perfusate; these data suggested control by a negative feedback process.

Enhanced splenic factor VIII production was observed with perfusion of CRM (+) hemophilia A plasma as compared with CRM (−) and von Willebrand's plasmas.

Similar but less striking enhancement of splenic factor IX production occurred with perfusion of CRM (+) hemophilia B plasma as compared with CRM(−) plasma.

The experiments summarized above demonstrated that antigenically detectable but nonfunctional coagulation factors VIII and IX are capable of inducing splenic factor VIII and IX production, respectively.

ACKNOWLEDGMENTS

The authors thank Ann C. Moynihan, Sharon L. Raymond, Joanne E. Kull and Carol J. Church for their technical assistance.

REFERENCES

1. Dodds, W. J. Storage, release and synthesis of coagulation factors in isolated perfused organs. Am. J. Physiol. 217:879–883, 1969.
2. Dodds, W. J. Organ perfusion studies in haemophilia. Proc. of the VIIth Congress of the World Federation of Haemophilia, Excerpta Medica (In press) 1971.
3. Dodds, W. J. Hepatic influence on splenic synthesis and release of coagulation activities. Science 166:882–883, 1969.
4. Rappazzo, M. E., and Hall, C. A. Transport function of transcobalamin II. J. Clin. Invest. 51: 1915–1918, 1972.
5. Dodds, W. J., and Hoyer, L. W. Coagulation activities in perfused organs. Regulation by addition of animal plasmas. Submitted for publication, 1972.
6. Hoyer, L. W. and Breckenridge, R. T. Immunologic studies of antihemophilic factor (AHF, Factor VIII): cross-reacting material in a genetic variant of hemophilia A. Blood 32:962–971, 1968.
7. Hoyer, L. W. and Breckenridge, R. T. Immunologic studies of antihemophilic factor (AHF, Factor VIII): II. Properties of cross-reacting material. Blood 35:809–820, 1970.
8. Roberts, H. R., Grizzle, J. E., McLester, W. D., and Penick, G. D. Genetic variants of hemophilia B: Detection by means of a specific PTC inhibitor. J. Clin. Invest. 47:360–365, 1968.
9. Bowie, E. J. W., Didisheim, P., Thompson, J. H. Jr., and Owen, C. A. Jr. The spectrum of von Willebrand's disease. Thromb. Diath. Haemorrh. 18:40–56, 1967.
10. Breckenridge, R. T., and Ratnoff, O. D. Studies on the nature of the circulating anticoagulant directed against antihemophilic factor: with notes on an assay for antihemophilic factor. Blood 20:137–149, 1962.
11. Larrieu, M. J., Caen, J. P., Meyer, D. O., Vainer, H., Sultan, Y., and Bernard, J. Congenital bleeding disorders with long bleeding time and normal platelet count. II. Von Willebrand's disease. Am. J. Med. 45:354–372, 1968.
12. Hoyer, L. W. Immunologic studies of antihemophic factor (AHF, Factor VIII). III. Comparative binding properties of rabbit and human antihemophilic factor. Blood 39:481–589, 1972.
13. Stites, D. P., Hershgold, E. J., Perlman, J. D., and Fudenberg, H. H. Factor VIII detection by hemagglutination inhibition: hemophilia A and von Willebrand's disease. Science 171:196–197, 1971.
14. Zimmerman, T. S., Ratnoff, O. D., and Powell, A. E. Immunologic differentiation of classic hemophilia (factor VIII deficiency) and von Willebrand's disease. J. Clin. Invest. 50:244–254, 1971.

DISCUSSION

CHAIRMAN SEEGERS: Thank you, Dr. Dodds. We'll now entertain questions. Are there any questions to ask Dr. Dodds at the present time?

E. J. W. BOWIE, Mayo Clinic, Rochester, Minnesota: I think that was a very fascinating study. The relationship, as you mentioned, of the stimulating activity of the liver to the possible mechanism of von Willebrand's disease is extremely intriguing. I wonder if you have done any work on the activity that stimulates factor VIII release from the spleen to see if it corrects the platelet retention in dogs with von Willebrand's disease?

DR. DODDS: We haven't done that yet, because it's quite difficult isolating the liver fraction and we have lots of experiments we want to use it for.

CHAIRMAN: I was wondering about one point and would like you to comment on it. You say that you can now get positive reactions to antibody tests quite commonly when you have a hemophilia-A. In other words, the molecule is there. About fifteen years ago we extracted hemophilia plasma with ether and developed factor VIII activity. Would that fit in with your findings now?

DR. DODDS: I think, Dr. Seegers, that's a very difficult question. Several years ago my former colleagues reported *in vivo* experiments in which they infused ether extracted serum and corrected factor IX deficiency. It would be impossible to speculate whether the experiments we performed are in any way related to the correction of hemophilic plasma by ether extraction.

CHAIRMAN: I have one more question along that same line. Do you think that factor IX production is dependent on Vitamin K?

DR. DODDS: I don't know. I think the whole prothrombin relationship with warfarin and Vitamin K is quite complex.

CHAIRMAN: Well, is there anything in your experiments that would lead you to believe that factor IX is not synthesized by a dependence on Vitamin K?

DR. DODDS: No. We have done many perfusions with warfarin which were not discussed today. Our results suggest that factor IX synthesis is dependent upon Vitamin K.

HEMOSTASIS IN THE ANIMAL KINGDOM

K. Laki*

Four mechanisms are known by which nature seals wounds: the three clotting systems (platelet system, transamidase system, cascade system) and vasoconstriction. All four systems are present in man. One or the other of the four systems operates in animals found at various levels of the evolutionary scale. The platelet system is exemplified in the horseshoe crab, the transamidase system in lobster. It will be illustrated with the interaction of fibrinogen and thrombin of various vertebrates and with the role of transamidase in atherosclerosis how comparative studies help to clarify various aspects of the clotting processes.

INTRODUCTION

When life started on earth in the primordial ocean, the primitive organisms obtained their nutrients from the surrounding fluid and discharged waste material into it. Eventually multicellular organisms developed large enough so that for these, exchange of molecules by diffusion became insufficient. To make sure that all portions of the animal remained close enough to the life supporting fluid, body cavities were developed to contain the fluid. For further development, it became important to prevent the accidental loss of this body fluid.

The evolution of body cavities, especially the closed ones in which the hemolymph circulates, permitted the animal to venture also onto dry land, an event which emphasized the importance of wound-sealing. Apparently, various mechanisms evolved to close a wound. The essential feature of these mechanisms is that injury triggers a series of events which lead to the sealing of the wound.

The accidental opening of the body cavity may be reduced or closed "simply" by muscular contraction. Lower invertebrates such as *Arenicola* (earthworm) utilize the contraction of the body wall musculature for wound-sealing. Vasoconstriction is more important in vertebrates where a closed vascular system has to be protected from fatal loss of blood.

In most instances, however, fatal loss of the body fluid is prevented by the formation of a "plug." The ingredients needed for the "plug formation" are either the circulating cells, or materials dissolved in the body fluid (plasma). The hemostatic cells (hemocytes) may just aggregate at the site of injury, or break up and release material which forms a fibrous network to plug the wound. When stasis of the body fluid is carried out by the elements of its plasma, a protein component of the plasma (coagulogen) becomes altered and comes out of solution in the form of long filaments, the entangled mesh of which plugs the wound. So far two basic mechanisms are known by which the clotting proteins become modified to form filaments. In one case, an activated enzyme connects the molecules of the clottable protein. In the other also, an activated enzyme splits off small parts of the clotting protein which comes out of solution and aggregates into long filaments.

The information available indicates that in their main features, the clotting mechanisms are similar in all the animal phyla. For example, the blood of the honeybee is reported to contain factors V, VIII, IX, X, XI and XII.[1]

The ancestry of these clotting mechanisms may be traced back to the metazoa. What complicates the picture of considering the various clotting mechanisms as being homologous is that in some species, especially in invertebrates, (there are some 70,000 insects), one or the other clotting factors predominate or are entirely absent. In the *Limulus,* plasmatic clotting is absent. It relies exclusively on the clotting protein released from circulatory cells. Lobster, on the other hand, converts its clotting protein

* National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland 20014.

of the plasma into a plug by an enzyme present in muscle tissues.

If we view hemostasis in the animal kingdom, we can discern four systems: (1) vasoconstriction, (2) thrombocyte system, (3) transamidase system, and (4) cascade system.

In man and in most vertebrates, all these four systems operate simultaneously, occasionally one or the other dominating the picture. It would be impossible for me to deal adequately with these systems in the animal kingdom; therefore, I will restrict myself to illustrating them with examples choosing those where our knowledge is on sufficiently solid ground.

THROMBOCYTE SYSTEM

For the illustration of the thrombocyte system, I would like to discuss briefly the clotting of the horseshoe crab *(Limulus polyphemus)*. This animal actually is not a crab but is related to the arachnid and thus belongs to the arthropods. The hemolymph of this arthropod contains one type of a circulatory cell called amoebocytes or hemocytes. In case of injury, these cells break up and release the clotting material. The amoebocytes are very sensitive to the ubiquitous endotoxin and when these cells come into contact with even trace amounts of this material, they disintegrate. The disintegrating cells release the clotting material which, in turn, in the presence of endotoxin, forms the clot.

Levin and Bang[2,3] discovered that if the blood of *Limulus* is drawn in the presence of N-ethyl maleimide, the cells are protected from the disruptive effect of the ever present endotoxin and can be separated from the plasma by centrifugation. The isolated cells may be lysed to obtain a cell-extract. This extract clots upon addition of endotoxin. Since the cell-free plasma does not clot and the clotting material in the cells represents the total clotting material, it is quite obvious that the amoebocytes alone are responsible for the clotting.

The time course of the clotting material isolated from the cells indicates an enzymatic process similar to clotting of fibrinogen by thrombin or transglutaminase. The endotoxin bringing about the clotting is viewed as an agent which activates a clotting enzyme or converts it to an active enzyme from a precursor.

Solum recently reported the purification of the clotting material which turned out to be a protein.[4] The molecular weight of this clottable protein appears to be about one-tenth of that of bovine fibrinogen.[4]

Vertebrates and a large number of invertebrates contain hemostatic cells. In some of the invertebrates, these cells function similar to what we see in *Limulus* blood. In others, the main purpose of the circulating cells is to form aggregates at the site of injury.

The thrombocytes and the platelets apparently carry out both of these functions. According to Levin and Bang[3] and Solum,[5] the hemocytes of the *Limulus* are the prototype of the thrombocytes and platelets. The thrombocytes are nucleated cells, while platelets are devoid of nucleus and are derived from the megakaryocytes. Platelets, similar to the hemocytes of *Limulus*, also contain fibrinogen or profibrin-like material which forms micro-clots when the platelets disintegrate.[6] The micro-clots generated from platelets are especially predominant in the so-called Shwartzman[7] reaction when the disintegrating platelets can cause fatal clots.

In the Shwartzman reaction, the disintegration of platelets is attributed to endotoxin. The involvement of endotoxin in platelet disintegration further emphasizes the analogy between the amoebocytes of the *Limulus* and the platelets. It appears that during the evolutionary process, the thrombocytic cells lost their sensitivity towards endotoxin, but when the sensitivity is regained, as in the generalized Shwartzman reaction, these cells behave as the hemocytes of the primitive invertebrates. The analogy of the platelets to the hemocytes of the invertebrates underlines the importance of the study of these primitive organisms for the understanding of the behavior of platelets in vertebrates and man.

TRANSAMIDASE SYSTEM

As an illustration of the operation of this system, I am citing the clotting of lobster's blood. Lobster *(Homarus)* is a crustacean, one of the most primitive representatives of the arthropods. The clot in the lobster's blood, in contrast to *Limulus* where it is strictly of cellular origin,

arises from a clottable protein dissolved in the plasma. The fibrinogen-like clottable protein in lobster's blood is clotted by a tissue enzyme, now recognized to be a transamidase.

The elucidation of the clotting of lobster's blood progressed in three stages. In 1954, Duchateau and Florkin[8] reported the clotting of lobster's "fibrinogen" by a muscle enzyme (coaguline). Later Lorand and co-workers[9] showed that liver extracts can clot lobster's blood and suggested that transamidase in the liver extract was responsible for the clot formation. That a transamidase can clot fibrinogen was demonstrated by Farrell and Laki[10] who clotted bovine fibrinogen by pure transglutaminase isolated from guinea pig liver.

In order to put the clotting of lobster's blood in perspective, I am going back to our discovery of the fibrin stabilizing factor. In 1945 we reported[11] that thrombin, under certain conditions, does not clot fibrinogen—only carries out some small alteration on the fibrinogen molecules.[12] It developed that the alteration was the splitting of some small peptides. The observation that the altered fibrinogen molecules (fibrin molecules) could be kept in solution suggested to us that it should be possible to bring a clot of purified fibrinogen into solution. Although, according to the literature, normal blood clot is insoluble in concentrated urea, we tried and found that urea readily dissolved a clot of purified fibrinogen. Not only did the clot dissolve in urea, but after dialyzing out the urea, the clot returned. Obviously, urea did not denature the fibrin molecules, only dispersed them. It was a simple matter for Lorand and me[13] to demonstrate that the clot will not dissolve in urea if we added a small amount of serum or plasma to our purified fibrinogen before clotting it.

Eventually it turned out from the clinical studies of Duckert and co-workers[14] that the absence of the plasma component which renders the clot insoluble in urea gives rise to a clotting defect. The clot stabilizing factor of plasma, or the Laki-Lorand factor, became known as Factor XIII.

We know now that Factor XIII is a precursor of transglutaminase, the role of which is to connect fibrin molecules in the clot with primary bonds.

The first indication that activated Factor XIII was a transamidase came when it was observed that ammonia was liberated in the stabilization reaction[15] and that the enzyme catalyzed the incorporation of amines into glutamine residues of fibrinogen.[16] Then came the demonstration that 4–6 γ-glutamyl-lysine bonds were formed in fibrin during clot stabilization.[17] From the work of Doolittle and others,[18] it became quite clear that the first two cross-bonds form near the C-terminal portion of the γ-chains of human and bovine fibrinogens. The muscle enzyme of lobster which clots lobster fibrinogen is also a transglutaminase and connects fibrinogen molecules with primary bonds similar to the transglutaminase of the plasma of vertebrates with the exception that the plasma enzyme connects fibrin molecules. However, as we have demonstrated,[10] transglutaminase can also clot fibrinogen molecules, not only fibrin molecules. In fact, it appears that in atherosclerosis, the disease of the arteries, transglutaminase does clot fibrinogen directly. We found[19] in experimentally-induced atherosclerosis in rabbits that the sclerotic plaques contain increased amounts of transglutaminase. It was also found by Shainoff et al.,[20] that sclerotic plaques also contained increased amounts of fibrinogen. However, Shainoff et al. did not demonstrate the presence of free fibrinogen, only increased amounts of the protein that released fibrinopeptides upon addition of thrombin. Microscopic examination reveals network structure in sclerotic plaques which people thought was a fibrin clot. It appears more likely, based on Shainoff's and our work, that the network structure in the sclerotic plaques is actually fibrinogen network. This suggests that in this diseased condition, the arterial tissue reverts to an ancient clotting habit operative in lobster's blood but which, under normal conditions, became outmoded in vertebrates.

CASCADE SYSTEM

The normal blood coagulation in man and in other vertebrates depends on half a dozen clotting factors of the plasma operating in a se-

quential manner, one factor activating the other until thrombin is formed. The process starts with the activation of the Hageman factor (Factor XII) by any surface other than the normal vascular endothelium. Then follows the activation of Factors XI, IX, VIII in succession. Finally, Factor X in the presence of phospholipids, and Factor V lead to the component that converts prothrombin. The details of prothrombin conversion are becoming increasingly clear. The reader is referred to an adjoining paper by W. H. Seegers for further information. In the presence of tissue factors, through Factor VII, a short cut is made for the activation of Factor X. The absence, or an abnormal form of any of these factors, may lead to bleeding disorders because of a delayed thrombin generation, not only in man, but in other animals, (hemophilia in dogs, for example). (For a review of the cascade system in vertebrates, the reader is referred to a recent article by Archer.)[21]

Here I intend to review a previous comparative study of the interaction of fibrinogen and thrombin isolated from various animals.[22]

It is now well established that when thrombin clots fibrinogen, a small portion (3 percent) of fibrinogen is split off in the form of two peptides referred to as peptides A and B by the limited proteolytic action of thrombin.[23] The fibrin molecules thus formed join together to a network structure, the fibrin clot.

The chemical composition of the peptides released from several different fibrinogens by the action of thrombin is now known. Table I shows the amino acid sequence of peptide A released from the fibrinogens of man, dog, ox, pig, sheep, goat, reindeer, rabbit and monkey (Rhesus).

The following is an examination of how the composition of these peptides may affect their release by thrombin.

For these experiments, thrombin and fibrinogen were isolated from eleven different animals. The fibrinogens were purified to give 96% clottable material.[24] For the preparation of thrombin, prothrombin was first adsorbed on barium sulfate from the plasma and through further purification, the prothrombin was converted to thrombin. The thrombin solutions were diluted

TABLE I

The amino acid sequence of these peptides was initiated in the author's laboratory with the determination of the sequence of bovine peptide A. The other sequences in the table originate from the work of Blombäck and co-workers and Doolittle and co-workers. (For references, see Atlas of Protein Sequence and Structure, pp. 165–170, 1967–68, M. O. Dayhoff and R. V. Eck, Natl. Biomed. Res. Foundation, Silver Spring, Md.)

	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
BOVINE	GLU	ASP	GLY	SER	ASP	PRO	PRO	SER	GLY	ASP	PHE	LEU	THR	GLU	GLY	GLY	GLY	VAL	ARG
EUROPEAN BISON	GLU	ASP	GLY	SER	ASP	PRO	ALA	SER	GLY	ASP	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
WATER BUFFALO	GLU	ASP	GLY	SER	ASP	ALA	VAL	GLY	GLY	GLU	PHE	(LEU.	ALA.	GLU.	GLY.	GLY.	GLY.	VAL)	ARG
SHEEP, GOAT	ALA	ASP	ASP	SER	ASP	PRO	VAL	GLY	GLY	GLU	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
PRONGHORN	ALA	ASP	GLY	SER	ASP	PRO	VAL	GLY	GLY	GLU	SER	(LEU.	PRO,	ASP.	GLY.	ALA,	THR,	GLY)	ARG
REINDEER	ALA	ASP	GLY	SER	ASP	PRO	ALA	GLY	GLY	GLU	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
MUNTJAK	(ALA.	ASP.	GLY.	SER.	ASP.	PRO.	ALA.	SER.	GLY.	GLU)	PHE	(LEU.	THR.	GLU.	GLY.	GLY.	GLY.	VAL)	ARG
SIKA DEER	ALA	ASP	GLY	(SER.	ASP.	PRO.	ALA.	SER.	SER.	GLU.	PHE.	LEU.	ALA.	GLU.	GLY.	GLY.	GLY.	VAL)	ARG
RED DEER	ALA	ASP	GLY	SER	ASP	PRO	ALA	SER	SER	ASP	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
ELK	(ALA.	ASP.	GLY.	SER.	ASP.	PRO.	ALA.	SER.	SER.	ASP)	PHE	(LEU.	ALA.	GLU.	GLY.	GLY.	GLY.	VAL)	ARG
LLAMA		THR	ASP	PRO	ASP	ALA	ASP	LYS	GLY	GLU	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
VICUNA		(THR.	ASP.	PRO.	ASP.	ALA.	ASP.	LYS.	GLY.	GLU)	PHE	(LEU.	ALA.	GLU.	GLY.	GLY.	GLY.	VAL)	ARG
CAMEL		THR	ASP	PRO	ASP	ALA	ASP	GLU	GLY	GLU	PHE	(LEU.	ALA.	GLU.	GLY.	GLY.	GLY.	VAL)	ARG
PIG			ALA	GLU	VAL	GLN	ASP	LYS	GLY	GLU	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
RAT			ALA	ASP	THR	GLY	THR	THR	SER	GLU	PHE	ILU	(ASP,	GLU.	GLY.	ALA,	GLY,	ILU)	ARG
HUMAN				ALA	ASP	SER	GLY	GLU	GLY	ASP	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
GREEN MONKEY				ALA	ASP	THR	GLY	GLU	GLY	ASP	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
RHESUS MONKEY				ALA	ASP	THR	GLY	GLU	GLY	ASP	PHE	LEU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
DONKEY				THR	LYS	THR	GLU	GLU	GLY	GLU	PHE	ILU	SER	GLU	GLY	GLY	GLY	VAL	ARG
RABBIT				VAL	ASP	PRO	GLY	GLU	SER	THR	PHE	ILU	ASP	GLU	GLY	ALA	THR	GLY	ARG
MINK				THR	ASN	VAL	LYS	GLU	SER	GLU	PHE	ILU	ALA	GLU	GLY	ALA	(ALA.	GLY)	ARG
BADGER				THR	ASP	VAL	LYS	GLU	SER	GLU	PHE	ILU	ALA	GLU	GLY	ALA	VAL.	GLY.	ARG
DOG				THR	ASN	SER	LYS	GLU	GLY	GLU	PHE	ILU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
FOX				THR	ASN	SER	LYS	GLU	GLY	GLU	PHE	ILU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
CAT				GLY	ASP	VAL	GLN	GLU	GLY	GLU	PHE	ILU	ALA	GLU	GLY	GLY	GLY	VAL	ARG
HORSE						THR	GLU	GLU	GLY	GLU	PHE	LEU	HIS	GLU	GLY	GLU	GLY	VAL	ARG

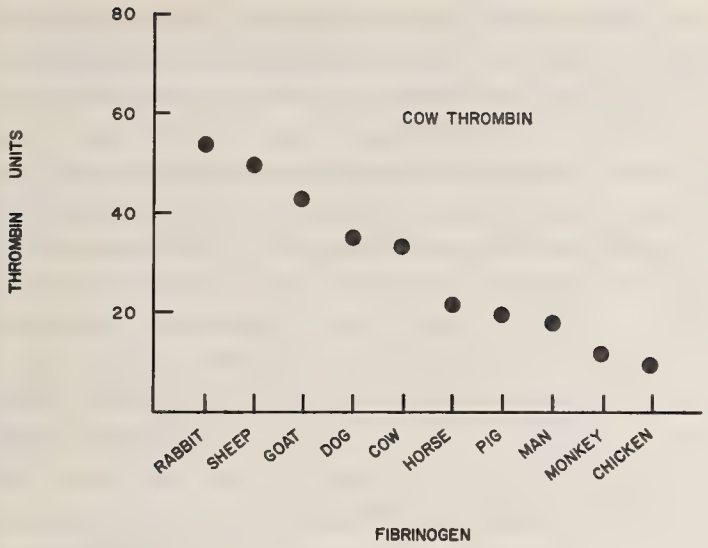


FIGURE 1.

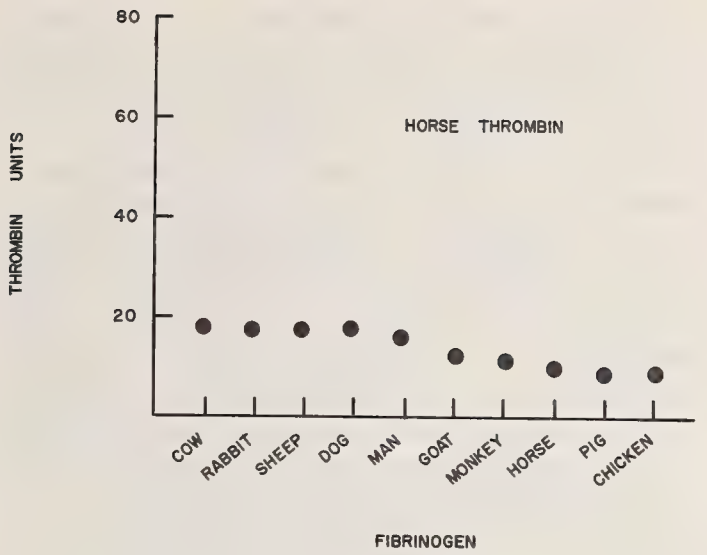


FIGURE 2.

The points in figures 1 and 2 represent the number of thrombin units found when cow and horse thrombin clotted the various fibrinogens (shown on the abscissa). Cow and human thrombins exhibited the greatest variations in clotting the various fibrinogens. In case of horse thrombin, the variation in the rate of clotting was very small.

to give clotting times, usually between 10 to 90 seconds. Solutions of the various fibrinogens to be clotted contained identical amounts of fibrinogen under identical conditions. The speed of the clotting reaction was measured from the addition of thrombin in the Klett colorimeter by following the development of turbidity. For the clotting time, we selected arbitrarily the time needed for the turbidity to reach about one-third of its final value. The clotting times were converted into thrombin units. A thrombin unit is the logarithm of the reciprocal of the clotting time. Since clotting follows the kinetics of a first order reaction, thrombin units are expressions of the velocity constants.

By means of the thrombin units, the various fibrinogens could be ordered according to the ease with which they interacted with a given thrombin (see Figures 1 and 2, and Table II). It is seen from Table II that chicken thrombin clotted the fibrinogens of rabbit, sheep, goat, pig, ox, horse, dog, monkey, and man faster than its own. Horse thrombin, on the other hand, clotted all of them with roughly the same speed (Figure 2). The variation of speed is not very great. Human thrombin was found to ex-

TABLE II.—*Thrombin*

The columns in this table show the fibrinogens in decreasing order corresponding to the speed with which these were clotted by a thrombin (given at the head of the column). The fibrinogens reacting fastest with the thrombin are at the top of the columns. Places where goat and sheep fibrinogen appear together are marked by circles. The entries marked with squares show those four instances where the clotting parameters of sheep and goat fibrinogen is separated by others.

	Monkey	Dog	Pig	Rabbit	Sheep	Goat	Cow	Man	Turkey	Chicken
Fibrinogen	Rabbit	Sheep O	Sheep □	Dog	Goat □	Dog	Rabbit	Cow	Rabbit	Goat O
	Dog	Goat O	Rabbit	Man	Rabbit	Man	Sheep O	Rabbit	Goat O	Sheep O
	Cow	Cow	Goat □	Cow	Man	Rabbit	Goat O	Sheep □	Sheep O	Rabbit
	Sheep O	Rabbit	Pig	Rabbit	Sheep □	Monkey	Dog	Dog	Chicken	Pig
	Goat O	Man	Cow	Sheep O	Dog	Cow	Cow	Goat □	Cow	Man
	Man	Dog	Dog	Goat O	Cow	Horse	Horse	Man	Pig	Cow
	Horse	Pig	Horse	Horse	Horse	Sheep □	Pig	Horse	Horse	Dog
	Monkey	Monkey	Man	Pig	Pig	Pig	Man	Pig	Dog	Horse
	Pig	Horse	Monkey	Monkey	Monkey	Goat □	Monkey	Monkey	Man	Monkey
	Chicken	Chicken	Chicken	Chicken	Chicken	Chicken	Chicken	Chicken	Monkey	Chicken

hibit the greatest variations; it clotted bovine fibrinogens ten times faster than chicken fibrinogen. With horse thrombin, the variation of speed was only about twofold. Thus, it would not make much difference for the horse if we replaced horse fibrinogen by chicken fibrinogen or any of the others. In contrast, such a substitution of fibrinogen in man could lead to serious complications. The rate of fibrinogen-thrombin interaction would become too fast or too slow depending on which fibrinogen is substituted for the human fibrinogen. For horse, an alteration (brought about by mutation) in the fibrinogen molecules would hardly affect the rate of clotting; but for humans, a similar alteration could mean a great difference in the speed of clotting. Apparently, human thrombin magnifies the differences which for horse thrombin appear insignificant.

The basic point that emerged from these experiments is that all the thrombins and fibrinogens tested were different. During evolution, both fibrinogen and thrombin underwent modifications.

I shall now attempt to correlate the data in Table II with those in Table I in order to see if some light can be shed on how evolution managed the fibrinogen-thrombin interaction.

In the arguments, actual rates of clotting are not involved. I am utilizing only the order in which the various fibrinogens can be arranged (Table II) according to the speed of their clotting brought about by a given thrombin.

Setting our sight on the fibrinogen first, the question naturally arises which portion of the fibrinogen molecule is responsible for the variation in the speed of clotting. Let us look first at the peptides which are released during clotting. Naturally we have to look for that portion of the peptides where the variations is the greatest —where the amino acid residues show the greatest variation. Since, in these experiments, the initial rates were measured, we have to examine peptide A which comes off during clotting earlier than peptide B.[25] In other words, it is peptide A that can be expected to have an influence on the initial rate.

A look at Table I shows the similarities of these peptides. All end at the C-terminal in arginine. This is, of course, due to the trypsin-like specificity of thrombin liberating these peptides by cleaving the bond between arginine and glycine. If we number the positions in peptide A starting with arginine, we can see that up to position 6, the residues in all the peptides are the same. The consistent presence of glutamic acid in position 6 is understandable. The main role of these peptides is to keep the fibrinogen molecules apart with their repelling negative charges. We expect this attribute of the peptides to be conserved during evolution. The great similarity of these peptides shows that they are really homologous and probably go back to a common ancestor.

Beyond position 11, the variations are becoming greater probably because up to this point, the essential requirements (charge in the proper position, complementariness to thrombin) have been fulfilled. The variation is the greatest at position 13. Thus we may conclude that the amino acid residue in this position has an effect on the rate of the cleavage of the bond 13 residues away.

This is not an entirely unexpected finding. When we used synthetic peptides containing a bond susceptible to thrombin action, we found that elongation of the peptide 3 to 4 residues beyond the bond split had a noticeable effect on the rate of splitting. For example, phe-gly-arg-amide (NH_3 is split off by thrombin) is hydroyzed faster than phe-arg-amide.[26] The action of thrombin apparently depends on what amino acid residue happens to be 13 residues away.

A corollary to this conclusion is that if we have identical residues at this point, the catalytic action of thrombin should be closely identical. Inspection of Table II reveals that in 6 out of 10 cases investigated, goat and sheep turn up together in the table. Six out of 10 thrombins clot goat and sheep fibrinogen with nearly the same rate. Thus, for 6 different thrombins, goat and sheep fibrinogen appear indistinguishable. This means that peptide A of goat and sheep fibrinogens must be similar at position 13. It is seen from Table I that both goat and sheep peptides contain valine at position 13.

Let us now examine the four instances in Table II where the thrombins are not strictly discriminating. With these four thrombins, the

rate of clotting of human, dog, rabbit and pig fibrinogen falls between those of sheep and goat. These thrombins apparently confuse valine with glycine, aspartic and glutamic acids at position 13. The question arises why do these thrombins confuse glycine, aspartic and glutamic acids with valine? The answers may be that residues located at positions further away may have an effect at position 13.

Inspection of Table I shows that what distinguishes the human, dog, rabbit and pig peptides from the others, is that at position 16 these do not have serine. Also, that human, dog, rabbit and pig do not have aspartic acid residues at position 18. We may assume that thrombin can discriminate strictly the amino acid residues at position 13 only if serine is located at position 16, or if aspartic acid is located at position 18.

For the purpose of further speculation, let us assume that it is position 18 which influences position 13 because the conclusions coming from this assumption may be checked. In order that aspartic acid at position 18 may influence the action of thrombin, the peptides at this position also would have to interact with thrombin. Since aspartic acid carries a negative charge, we may attribute the influence of aspartic acid at position 18 to a positively charged group on the thrombin molecule.

In the case of chymotrypsin, it is well established that there is indeed a positively charged group on the molecule that influences the activity of the enzyme. This group has been identified as the N-terminal isoleucine.[27-29] According to current views, the amino group of isoleucine forms a salt bridge with the aspartic acid residue located next to the serine residue of the active site. This "activation salt bridge" is supposed to regulate the activity of the enzyme. Thrombin also has N-terminal isoleucine.[30] The aspartic acid at position 18 may then be assumed to interfere with this "activation salt bridge" by competing for the amino group of the terminal isoleucine.

From these considerations, we may conclude that if peptide A contains aspartic acid residue at position 18, this residue, by forming an electrostatic (or hydrogen bond) with the N-terminal isoleucine of thrombin, enables it to discriminate among the different residues that occur at position 13, probably because in such a case, the peptide chain is tied down at position 18. These considerations suggest that peptide A may be attached to thrombin at positions 13, 18, and perhaps at 16, as well.

The dissociation of the α-amino group of the N-terminal isoleucine in chymotrypsin is known to influence the acylation step of this enzyme. Because of the great similarity of thrombin to trypsin and chymotrypsin,[31-32] we may tentatively conclude that the changes brought about in peptide A at position 18 during evolution affect the acylation step in the thrombin-fibrinogen interaction. At the beginning, our assumption was that the structure of peptide A has an effect on the rate with which thrombin splits off this peptide from fibrinogen. This assumption led to interesting consequences indicating that it was justified.

During evolution, thrombin also underwent modifications. This is discernible from the qualitative and the quantitative response with which thrombins adjust to the changes in fibrinogen: (1) different thrombins respond differently to changes taking place in fibrinogen; (2) also, different thrombins magnify these changes to a different extent.

SUMMARY

The experiments discussed show that during evolution both fibrinogen and thrombin have changed. When the different rates of clotting were compared to the amino acid sequence of the peptides released in the clotting reaction, a correlation could be detected between the speed of reaction and the amino acid residues at a certain locus in the peptides. The observations suggest that the peptidylation (acylation) of thrombin in the enzyme substrate interaction is influenced by the amino acid residues placed at a given position by the evolutionary change.

REFERENCES

1. Gilliam, M., and Shimanuji, H. Progress report: studies on honey bee blood. Amer. Bee J. 107:256, 1967.
2. Levin, J., and Bang, F. B. A description of cellular coagulation in the Limulus. Bull. Johns Hopkins Hosp. 115:337, 1964.

3. Levin, J., and Bang, F. B. Clottable protein in Limulus; its localization and kinetics of its coagulation by endotoxin. Thromb. Diath. Haemorrh. 19:186, 1968.
4. Solum, N. O. Some characteristics of the clottable protein of Limulus polyphemus blood cells. Thromb. Diath. Haemorrh. 23:170, 1970.
5. Solum, N. O., and Lopaciuk, S. Bovine platelet proteins. II. Purification of platelet fibrinogen. Thromb. Diath. Haemorrh. 21: 1969.
6. Solum, N. O., and Lopaciuk, S. Bovine platelet proteins. III. Some properties of platelet fibrinogen. Thromb. Diath. Haemorrh. 21: 1969.
7. Pappas, G. D., Ross, M. H., and Thomas, L. Studies on the generalized Shwartzman reaction. VIII. The appearance, by electron microscopy, of intravascular fibrinoid in the glomerular capillaries during the reaction. J. Exp. Med. 107:333, 1958.
8. Duchateau, G., and Florkin, M. Coagulation of the blood of arthropods. IV. Fibrinogen and muscle coagulin of the lobster. Bull. Soc. Chim. Biol. 36:295, 1954.
9. Lorand, J., Urayama, T., and Lorand, L. Transglutaminase as a blood clotting enzyme. Biochem. Biophys. Res. Commun. 23:828, 1966.
10. Farrell, J., and Laki, K. Clotting of bovine fibrinogen by liver transamidase. Blood 35:804, 1970.
11. Laki, K., and Mommaerts, W. F. H. M. Transition of fibrinogen to fibrin as a two-step reaction. Nature 156:664, 1945.
12. Laki, K. The polymerization of proteins: the action of thrombin on fibrinogen. Arch. Biochem. Biophys. 32:317, 1951.
13. Laki, K., and Lorand, L. On the solubility of fibrin clots. Science 108:280, 1948.
14. Duckert, F., Jung, E., and Shmerling, D. H. A hitherto underscribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. Thromb. Diath. Haemorrh. 5:179, 1960.
15. Loewy, A. G. Turnover of Clotting Factors. In: *Fibrinogen and Fibrin.* F. K. Schattauer, Verlag, Stuttgart p. 109, 1964.
16. Lorand, L., and Ong, H. H.: Studies on fibrin crosslinking. Nature of the acceptor groups in transpeptidation. Biochem. Biophys. Res. Commun. 23:188, 1966.
17. Pisano, J. J., Finlayson, J. S., Peyton, M. P., and Nagai, Y. ϵ- (γ-glutamyl) lysine in fibrin: lack of crosslink formation in factor XIII deficiency. Proc. Natl. Acad. Sci. USA 68:770, 1971.
18. Chen, R., and Doolittle, R. F. Isolation, characterization, and location of a donor-acceptor unit from cross-linked fibrin. Proc. Natl. Acad. Sci. USA 66:472, 1970.
19. Benko, A., and Laki, K. Studies on the clot stabilizing enzyme in aorta of rabbits under normal conditions and after cholesterol feeding. Biochem. Biophys. Res. Commun. 31:231, 1968.
20. Shainoff, J. R., Nogueira, W. P., Hazard, J. B., Bumbus, F. M., and Page, I. H. Fibrinogen content of atherosclerotic aortas. Biophys. J. 8:A-42, 1968 (Abstract).
21. Archer, R. K. in *The Haemostatic Mechanism in Man and Other Animals;* The Proceedings of a Symposium Held at the Zoological Society of London on the 4th and 5th December, 1969. Edited by R. G. MacFarlane, Academic Press, p. 121, 1970.
22. Chandrasekhar, N., and Laki, K. Evolution and the Fibrinogen-Thrombin Interaction. In: *Fibrinogen.* Edited by K. Laki, Marcel Dekker, Inc., New York, p. 117, 1968.
23. Laki, K. Introduction and Summary. In: *Fibrinogen.* Edited by K. Laki, Marcel Dekker, Inc., New York, p. 1, 1968.
24. Laki, K. The polymerization of proteins; the action of thrombin on fibrinogen. Arch. Biochem. Biophys. 32:317, 1951.
25. Laki, K., and Gladner, J. A. The chemistry and physiology of the fibrinogen-fibrin transition. Physiol. Rev. 44:127, 1964.
26. Levin, Y., Folk, J. E., Gladner, J. A., and Laki, K. Studies on the specificity of thrombin. Fed. Proc. 19 (Part 1) :332, 1960 (Abstract).
27. Labouesse, B., Oppenheimer, H. L., and Hess, G. P. Conformational changes accompanying the formation of chymotrypsin-substrate complexes. Evidence for the involvement of an N-terminal α-amino group in the activity and the conformation of the enzyme. Biochem. Biophys. Res. Commun. 14:318, 1964.
28. Bender, M. L., and Kezdy, F. J. The current status of the α-chymotrypsin mechanism. J. Amer. Chem. Soc. 86:3704, 1964.
29. Fersht, A. R., and Requena, Y. Mechanism of the α-chymotrypsincatalyzed hydrolysis of amides. pH dependence of k_c and K_m. Kinetic detection of an intermediate. J. Amer. Chem. Soc. 93:7079, 1971.
30. Magnuson, S. Structural aspects of thrombin and prothrombin. In: *Structure-Function Relationship of Proteolytic Enzymes.* Edited by P. Desnuelle, H. Neurath, M. Otteson, Academic Press, New York, p. 138, 1970.
31. Gladner, J. A., and Laki, K. The active site of thrombin. J. Amer. Chem. Soc. 80:1263, 1958.
32. Curragh, E. F., and Elmore, D. T. Kinetics and mechanism of catalysis by proteolytic enzymes. 2. Kinetic studies of thrombin-catalyzed reactions and their modification by bile salts and other detergents. Biochem. J. 93:163, 1964.

DISCUSSION

Questioner: Dr. Laki, what implications do differences in fibrinogen and so forth have on using animal models for testing clot promotion or retarding drugs?

Dr. Laki: Would you repeat that?

CHAIRMAN SEEGERS: If I understood him correctly, he asked what these differences you have picked out have to do with the use of drugs for controlling coagulability in man?

DR. LAKI: Well, the drugs we use to control coagulability are acting on steps preceding this one. However, I am quite sure that some day we'll be able to influence this aspect of the reaction series.

ROGER POWELL, NIH, Bethesda, Md.: You observed the transaminase reaction in the lobster to be very similar to that in man. Is there any experience to show what it is in non-human primates?

DR. LAKI: I don't think anybody has tried it yet. I'm sure it will come.

BLOOD GROUPS OF APES AND MONKEYS: HUMAN AND SIMIAN TYPES

J. Moor-Jankowski*

INTRODUCTION**

Until ten years ago little was known about the blood groups of man's closest relatives, the nonhuman primates. A thorough review by Franks[1] published in 1962 cites only 66 articles for the period beginning 1911, while some of the reports were on a single animal only, and many of the results claimed have since been disproved.

This paucity of information was in contrast to the great amount of data available for cattle and mice: the former were investigated mainly because of the commercial importance of the information for stockbreeders, while the latter were ideal for experimental work because of their small size and ease of breeding. It is somewhat ironical that the convenience of using mice took precedence over their taxonomic distance from man.

The lack of information on blood groups of apes and monkeys may be ascribed to their limited availability prior to 1960 for basic research, and to the lack of appropriate handling methods for repeated bleeding and immunization. Moreover, the serological techniques in use were complicated. Prior to the introduction of the absorption and titration techniques of Wiener and Moor-Jankowski[2,3] blood typing of simians entailed the difficult preparation of unstable and low-titered eluates from human red cells treated with typing sera, a method introduced by Landsteiner and Miller in 1925.[4] The establishment of our own Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) and access to animals maintained by the U. S. Air Force and by some of the primate centers resolved the first difficulty by making an adequate number of animals available for our work. This, in turn, enabled us to develop our selective absorption technique[2] which can readily be carried out provided that a sufficient number of test animals is available. Moreover, in the meantime, new methods of husbandry and handling simians for immunological experiments have been developed in our laboratory.

Among the 66 articles cited in the 1962 review by Franks,[1] 16 were by Alexander S. Wiener, whose interest in simians predates his epoch-making discovery of the Rh factor. In 1962, Dr. Wiener and the author joined forces for a long-term study of blood groups of nonhuman primates. The results obtained during the first phase of our research program led us to establish the concept of *human-type, simian-type* and *cross-immune-type* blood groups in primates including man. The definition of these three categories, at which we arrived in 1964, has been most conducive for planning and advancement of further research.

Human-type blood groups[5] of primate animals are determined with reagents originally prepared for blood group tests in man, after suitable modification of these reagents by absorption, dilution or elution, in order to eliminate the interfering reactions of non-specific heteroagglutinins. The human-type blood groups are the *homologues* in simians of blood groups of man. So far homologues of human A–B–O, M–N, Rh–Hr, I–i and Lewis systems have been found in all species of apes, to a lesser extent in Old World monkeys, and to a limited degree in New World monkeys.

Simian-type blood groups[6] are determined using reagents prepared by immunization with red cells of apes and monkeys. That simian-type blood groups may be present not only in simians but also in man has been demonstrated by Landsteiner and Wiener's discovery of the Rh factor in man,[7] detected by antisera of rabbits im-

*Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP), New York University School of Medicine, New York
**Supported by USPHS, NIH, grant GM 12074.

munized with rhesus red cells. Some of the numerous simian-type blood groups found by us have been shown to be *analogues* of blood groups in man. Thus, in chimpanzees, we have found analogues of the M–N and Rh–Hr systems, and analogues of the M–N system also appear to exist in Old World monkeys.

Cross-immune-type blood groups in primates are determined with reagents prepared by immunization of simians with red cells of other primate species, including the use of human red cells for immunization of simians. The term "cross-immune" has been introduced by us to stress the special immunological nature of reactions between closely related species, which differ from heteroimmune reactions yet do not fall into the isoimmune category.[6,8]

METHODOLOGY

Our investigations of blood groups of apes and monkeys resulted from our desire to obtain new tools for the study of immunological reactions and immunogenetics in man. Therefore, in our studies on simians we use the same methods that have proved useful for studying the blood groups of man, rather than apply techniques which have been developed for studies of blood groups in mice (dextran method), and cattle (test for hemolysis). Other workers who had initially used the dextran method for their work on monkeys[9] have more recently abandoned that method and rallied to our concept.[10]

For testing blood of primates we use the classic methods of human blood typing, namely, the saline agglutination method, the antiglobulin test, the agglutination test with red cells treated with the proteolytic enzyme ficin, and the antiglobulin test with ficinated red cells. Moreover, in order to avoid tedious absorptions and to obtain reagents of the highest specificity we rely primarily on reagents produced by immunization of simians. We scarcely ever use sera prepared in rabbits (aside from the standard anti-M and anti-N reagents prepared for typing human blood), even though rabbit antisera have given good results in the hands of Owen and Anderson[11] and in some of our own early work.[12] Other reagents used by us are extracts of seeds (so-called lectins) such as *Dolichos biflorus* (anti-A_1), *Ulex europeus* (anti-H) and *Vicia graminea* (anti-N), and extracts of snails, notably *Helix pomatia* (anti-A). Lectins and snail agglutinins have the advantage of the absence of interfering nonspecific agglutinins.

To avoid pitfalls due to heteroagglutinins, and because many of our sera are not yet standardized and, finally, because the work involves testing of numerous species, our results are carefully controlled by the following techniques:

multiple comparative titrations;
use of standard panels of red cells;
use of reagents of high titers, avidity and specificity;
multiple testing to prove reproducibility;
"blind" reading of the tests to insure objectivity.

These precautions have helped us to avoid errors found in some of the earlier and even in some more recent reports.[13] It cannot be emphasized too strongly that we never rely on one-tube tests and apply the same standards to blood typing of simians as are considered essential for the application of blood grouping in clinical and legal medicine. It would be considered reprehensible to transfuse blood to a patient or offer an opinion in a medicolegal case on the base of a single test. This extreme care is necessary in our laboratory because some of our results on primates have been applied for the development of clinical procedures such as cross-circulation and organ transplantation, while other procedures are directly translated from experimentation in simians to clinical procedures in man. To be sure, interesting results have been obtained in recent work on rhesus blood groups using reagents of minimal titer in one-tube tests.[14,15] However, in our own laboratory low-titered antisera are considered only an indication for further courses of immunization in order to produce more potent reagents.

RESULTS

For detailed information on the results obtained, the readers are directed to our chapters in two recent monographs.[16,17] Here we will present in tables I–VIII a schematic summary of the most essential findings.

(1) As recently as 1967,[18] at the convention

TABLE I.—*Historical Synopsis*

1900	A–B–O	Man	(Landsteiner)
1911	A	Chimps	(V. Dungern & Hirszfeld)
1925	A–B–O	Apes	(Landsteiner & Miller)
	A–B–O	Indication in monkeys	(Landsteiner & Miller)
1937–1938	A–B–O	Monkey secretions	(Wiener)
	M–N	Chimps & monkeys	(Wiener & Landsteiner)
	Rhesus factor	Man	(Landsteiner & Wiener)
1940–1960	A few studies on limited number of simians		(Various investigators)
1963–1966	A–B–O, M–N, Rh–Hr	Numerous simian species	(Wiener & Moor-Jankowski)
1965–1972	Simian blood types	Chimps, gibbons, baboons, cynopithecus n., macaques	(Moor-Jankowski & Wiener)
1971		Rhesus	(Stone et al.)

TABLE II.—*Comparison of the A–B–O Blood Groups in Man and Apes Arranged in Order of Similarity to Man*

Species	Blood groups present	Subgroups of A	
Man	O, A, B, AB	A_1, A_2, etc.	Racial differences in distribution
Gibbon	A, B, AB	A_1, A_2 like man	Racial differences in distribution
Dwarf chimpanzee	A	A_1, like man	Racial differences in distribution
Chimpanzee	O, A	$A_{1,2}$	
Orangutan	A, B, AB	All A_1-like	
Gorilla			
Mountain	A	Undetermined	Red cells lack A, B and H
Lowland	B		Grouping by saliva only

TABLE III.—*Human-Type A–B–O Blood Groups in Anthropoid Apes*

Species		Blood group distribution in animals tested				
		O	A	B	AB	Totals
Chimpanzees						
Pan troglodytes	ASW & M-J	37	251	0	0	288
	Other authors	23	135	0	0	158
Pan paniscus	Other authors	1	5	0	0	6
	Other authors	2	15	0	0	17
Gibbons						
Hylobates lar	ASW & M-J	0	10	24	22	56
	Other authors	0	2	10	2	14
Hylobates hoolock	ASW & M-J	0	1	0	0	1
Siamangs						
Symphalangus brachytanites	ASW & M-J	0	0	2	0	2
Orangutans						
Pongo pygmaeus	ASW & M-J	0	22	1	3	26
	Other authors	0	12	11	11	34
Gorillas						
Gorilla gorilla gorilla	ASW & M-J	0	0	14	0	14
	Other authors	0	0	9	0	9
Gorilla gorilla berengei	Other authors	0	2	0	0	2

of the American Society of Clinical Pathologists, the effect of A–B–O blood group incompatibility on the survival of renal transplants in man was discussed. It was found that when donors of an incompatible blood group were used, the grafts did poorly in comparison to A–B–O compatible grafts. Thus, the life span of patients who received the blood group incompatible grafts was shortened. The point is that this kind of human experimentation has been obviated because analogous experiments can and should be carried out in nonhuman primates.

(2) Six years after our report on baboon blood groups,[2,3] Hume *et al*[19] used blood group compatible baboons for cross-circulation with patients in hepatic coma. This treatment has since been used by a number of workers who in several cases succeeded in sustaining their patients. However, the procedure used was discouragingly complicated, time consuming and expensive, because the animals, prior to receiving

TABLE IV.—*Human-Type A–B–O Blood Groups of Old World Monkeys (Catarrhina)*

Species	Blood group distribution in the animals tested				
	O	A	B	AB	Totals
Baboon					
Papio cynocephalus	0	18	20	22	60
Papio anubis	0	53	56	65	174
Papio ursinus, South Africa	0	4	59	26	89
Papio papio, Senegal	2	27	93	66	188
Papio (species undetermined)	1	42	66	65	173
Drills					
Mandrillus leucophaeus	0	4	0	0	4
Geladas					
Theropithecus gelada	18	0	0	0	18
Celebes black apes					
Cynopithecus niger	1(?)	23	2	0	26
Patas monkeys					
Erythrocebus patas	0	26	0	0	26
Vervet monkeys					
Cercopithecus pygerythrus	0	7	1	0	8
Macaques					
Macaca mulatta	0	0	46	0	46
Macaca fascicularis	1	23	19	19	62
Macaca nemestrina	3	0	7(?)	0	10
Macaca speciosa	0	0	4	0	4

TABLE V.—*Human-Type A–B–O Blood Groups of New World Monkeys (Platyrrhina)*

Species	Blood group distribution in the animals tested				
	O	A	B	AB	Totals
Spider monkeys					
Ateles marginatus	0	3	0	0	3
Ateles paniscus	0	1	0	0	1
Ateles ater	0	6	0	0	6
Ateles cucullatus	1	0	4	0	5
Capuchin monkeys					
Cebus albifrons	1	0	3	0	4
Squirrel monkeys					
Saimiri sciurea	1	3	0	0	4
Marmosets					
Various species	0	31	0	0	31

TABLE VI.—*Comparison of the M–N Blood Groups in Man and Apes Arranged in Order of Similarity to Man*

Species	Blood groups present	
Man	M, N, MN	Racial differences in distribution
Gibbon	M, N, MN	Racial differences in distribution
Dwarf chimpanzee, Chimpanzee	M, MN	
Gorilla	N, MN	
Orangutan	M	

human blood, were exsanguinated under hypothermia while on cardiopulmonary bypass. On the basis of our experience with blood grouping of baboons and the knowledge of their immunological reactions, a much simpler procedure was devised by Goldsmith[20] who prepared the animals for cross-circulation by simple exchange transfusion as in human erythroblastotic babies. Availability of our antisera made possible differential agglutination tests to determine the degree of blood exchange, which was shown to be virtually complete, thus ensuring the safety of the cross-circulation procedure.

(3) The information provided by our work during the last decade has resulted in the increased use of primate animals in experimental surgery requiring blood transfusion and priming of the heart-lung machine. Moreover, the large number of experiments using apes and monkeys as models for organ transplantation, and the simian-to-man transplantation studies became possible only after the blood groups of these animals were determined in our investigations.

SUMMARY

New testing methods have been developed and a large amount of information has been accumulated during the last decade of study, together with Alexander S. Wiener, on blood groups in apes and monkeys. A new concept has been established of human-type and simian-type blood groups in primates, including man.

Human-type blood groups, homologues of those of man, are determined with reagents originally prepared for testing human blood. Reliable tests can be made for the human systems A-B-O and H, M-N, Rh-Hr, I-i and Lewis in apes and in Old World monkeys *(Catarrhina)*. The New World monkeys *(Platyrrhina)* appear immunologically too distant from man to permit reliable tests for human blood types.

Simian-type blood groups, analogues of those of man, are determined with reagents produced by immunization with red cells of apes and monkeys.

At present chimpanzee is among the animal species whose blood groups are best known. The investigations on gibbons, baboons and rhesus are progressing rapidly.

We have limited our simian-type blood group studies to apes and Old World monkeys. However, Gengozian is presently determining the simian types of marmosets.

Our methods of testing for human-type and simian-type blood groups are the same as used

TABLE VII.—*Comparison of the Rh–Hr Blood Types in Man and Apes Arranged in Order of Similarity to Man*

Species	Factors present	Human Rh_o types	Simian Rh analogues
Man	Rh_o, Rh', Rh'', Hr', Hr'', etc.	Rh_o pos., Rh_o neg., Rh_o variants	
Gorilla	Rh_o, Hr'	Rh_o pos., Rh_o neg. (variants?)	
Chimpanzee	Rh_o, Hr'	All Rh_o pos.	CcEF
Gibbon	Hr'	All Rh_o neg.	
Orangutan	Rh_o, Hr'	All Rh_o pos., weaker than man	

TABLE VIII.—*Simian-Type Blood Groups in Apes and Monkeys*

Species		Simian-type blood factors
Chimpanzee	V-A-B system	V^c, A^c, B^c
	C-E-F system	C^c, c^c, E^c, F^c, L^c
	Others	G^c, H^c, I^c, J^c, K^c, M^c, "Lindsay", "Mandy", "Ella", "Chuck"
Gibbon		A^g, B^g, C^g and 5 others presently under study
Baboon		A^P, B^P, C^P, D^P, E^P, G^P, H^P, I^P, and 2 others presently under study
Rhesus monkey		A^{rh}, B^{rh}, C^{rh}, D^{rh}, E^{rh}, F^{rh}, G^{rh}, H^{rh}, I^{rh} and 4 others presently under study
Cynomolgus monkey		5 blood factors, presently under study
Celebes black ape		A^{ba}

in human blood typing, namely, the saline agglutination, the antiglobulin, the enzyme-treated red cell, and the enzyme-treated red cell antiglobulin method, as well as inhibition tests for A-B-H and Lewis substances in saliva. All antisera have to undergo careful absorptions to prevent false positive reactions due to nonspecific antibodies present in the majority of the sera.

The information accumulated through our work during the last decade has resulted, among others, in the increased use of primate animals in experimental surgery requiring blood transfusion and priming of the heart-lung machine. All the chimpanzees and baboons used as organ donors for human patients throughout the world since 1964, as well as most of the primate animals used in cross-circulation of patients in hepatic failure, have been tested in our laboratory.

REFERENCES

1. FRANK, D.: The blood groups of primates. Symp. Zool. Soc., London, 1962, vol. 10, p. 221 (Zool. Soc. London).
2. WIENER, A. S. and MOOR-JANKOWSKI, J.: Blood groups in anthropoid apes and baboons. Science, 142:67, 1963.
3. MOOR-JANKOWSKI, J., WIENER, A. S. and GORDON, E. B.: Blood groups of apes and monkeys. I. The A-B-O blood groups in baboons. Transfusion, 4:92, 1964.
4. LANDSTEINER, K. and MILLER, C. P.: Serological observations on the relationship of the bloods of man and anthropoid apes. Science, 61:492, 1925.
5. MOOR-JANKOWSKI, J., WIENER, A. S., and ROGERS, C. M.: Human blood group factors in non-human primates. Nature, 202:663, 1964.
6. MOOR-JANKOWSKI, J. and WIENER, A. S.: Simian-type blood group factors in non-human primates. Nature, 205:369, 1965.
7. LANDSTEINER, K. and WIENER, A. S.: An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc. Soc. exp. Biol., N.Y. 43:223, 1940.
8. MOOR-JANKOWSKI, J., WIENER, A. S., KRATOCHVIL, C. H. and FINEG, J.: Chimpanzee blood groups: Demonstrated with isoimmune and cross-immune sera. Int. Arch. Allergy, 29:397, 1966.
9. HIROSE, Y., and BALNER, H.: Red cell iso-antigens of rhesus monkeys. Blood, 34:661–681, 1969.
10. BALNER, H.: personal communication, 1971.
11. OWEN, R. D., and ANDERSON, D. R.: Blood groups in rhesus monkeys. Ann. N. Y. Acad. Sci., 97:4, 1962.
12. MOOR-JANKOWSKI, J., WIENER, A. S., GORDON, E. B. and GUTHRIE, C. B.: Simian blood groups. A 'new' blood factor A^{ba}, of Celebes black ape red cells demonstrated with rabbit antisera. Folia primat, 3:245, 1965.
13. BUETTNER-JANUSCH, J.: Antihuman sera and primate erythrocytes. Ann. N. Y. Acad. Sci. 97: 1962.
14. DUGGLEBY, C. R. and STONE, W. H.: Immunogenetic studies of rhesus monkeys. I. The G blood group system. Vox Sang., 20:109–123, 1971.
15. DUGGLEBY, C. R., BLYSTAD, C. and STONE, W. H.: Immunogenetic studies of rhesus monkeys. I. The H, I, J, K and L blood group systems. Vox Sang., 20:124–136, 1971.

16. Moor-Jankowski, J. and Wiener, A. S.: Red cell antigens of primates; in *Fiennes* Pathology of simian primates, Part I, pp. 270–317, Karger Basel, 1972.
17. Wiener, A. S. and Moor-Jankowski, J.: Blood groups of nonhuman primates and their relationship to the blood groups of man; in *Chiarelli* Comparative genetics in primates pp. 71–95, Academic Press, London/New York, 1971.
18. Tung, K. S., Deadhar, S. D. and Nakamoto, S.: The effect of A-B-O blood group incompatibility on renal transplantation in man. Annual Meeting of the American Society of Clinical Pathologists, Chicago, Ill., 1967.
19. Hume, D. M., Gayle, W. S. and Williams, G. M.: Cross-circulation of patients in hepatic coma with baboon partners having human blood. Surg. Gyn. Obstet., 128:495, 1969.
20. Goldsmith, E. I., Moor-Jankowski, J., Wiener, A. S.; Allen, F. H. and Hirsch, R.: Exchange transfusion of nonhuman primates with human blood. A program for preparation of cross-circulation partners in hepatic failure. *Medical Primatology*, pp. 80–91, 1970, Edited by E. I. Goldsmith and J. Moor-Jankowski. Karger, Basel/New York, 1971.

DISCUSSION

C. J. Stormont, University of California, Davis, Calif.: Dr. Moor-Jankowski, where do you and Dr. Wiener do your work? Is Dr. Wiener doing most of the work at his own laboratory, or do you both work at the medical school?

Dr. Moor-Jankowski: In the medical school. Dr. Wiener receives NIH support for his large laboratory at New York University Medical Center devoted specifically to typing of non-human primates. In addition, we have the Laboratory for Experimental Medicine and Surgery in Primates, sponsored by Associated Medical Schools of Greater New York. If anybody is interested in preservation of red cells, I presume we are the only laboratory in the world with methods and possibilities for liquid freezing, i.e., freezing primate red cells in liquid nitrogen.

Dr. Stormont: You were talking about the complete interchange of blood between chimpanzees and man. Have you heard of any recent experiment where lower animal species' blood has been used to transfuse man? For an example, Dr. Wiener and Dr. Unger had one patient, an aged lady, who had auto-immune hemolytic disease. Finally she couldn't tolerate human blood and had developed antibodies for practically every antigen system known in man. As a last resort, they asked me to ship them some cattle blood from a healthy animal immediately. I sent them some and they transfused her with the blood. It kept her alive eight weeks beyond her expected death time.

Dr. Moor-Jankowski: I haven't heard of this, which is quite interesting because I work so closely with Dr. Wiener. In light of what is being done now with non-human primates, I think this should be published.

SHEEP BLOOD COAGULATION VALUES

M. L. Povar*

Twenty-five mature sheep were selected at random from a flock of about 100 animals used for artificial lung test and evaluation. Coagulation values were established for these 25 animals and compared with those of normal human adults. While most values were comparable to man, certain significant differences were found. The hemostatic system of the sheep showed a marked decreased fibrinolytic activity and an increased platelet number and adhesiveness. These factors were probably responsible for the commonly observed lack of excessive bleeding in sheep. The increases noted in factor V and VIII levels were not great enough to certainly suggest a tendency toward thrombosis.

INTRODUCTION

If sheep are to be used effectively as animal models in the evaluations of artificial lungs more has to be known about the coagulability of sheep blood. A study conducted at Brown University has already provided some information on the subject.[1] The coagulation profile of sheep blood was determined for a group of 25 mature western Rambouillet-Columbia cross non-bred ewes that were part of a flock used by the Artificial Lung Test and Evaluation Facility (Table I) .

Some of the data on the blood clotting factors of sheep were markedly different from the human blood samples used as controls. Those factors believed to be of special importance will be discussed.

The methods and procedure for establishing the profile were described in detail in the previous publication. It should be emphasized, however, that blood samples were taken from the same and different individuals over a period of several weeks. This was done to increase the reliability of some of the data collected, because Morag et al. have demonstrated that coagulation time varied somewhat from one individual sheep to another and among different breeds. Small but significant day-to-day and week-to-week differences were also noted. They also found that prothrombin time varied significantly in sheep of different origins and in samples drawn on different days and weeks from the same sheep.[2]

* Brown University, Providence, Rhode Island.

RESULTS AND DISCUSSION

In the Brown University sheep, the clotting time was similar to that of man. Whole blood clotting time in glass ranged from 5 to 15 minutes, while the same test in plastic ranged from 5 to 57 minutes. These results differ markedly from those of Didisheim et al. who found that clotting time in plastic was equal to that in glass.[3] However, their report was based on only one measurement in one sheep.

Whole blood lysis did not occur in a period of 24 hours in the sheep blood while the human blood controls varied from 0–20% in 24 hours. The uniform total lack of spontaneous lysis suggests very low fibrinolytic activity.

Factor V values ranged from 160–800% of control compared to the human blood ranging from 60–140%.

Factor VIII values for sheep ranged from 440–1650% of control compared to human blood which ranged from 50–200% of control. These markedly higher Factor V and VIII values for the sheep might suggest some "increased tendency to coagulate" as some authors consider elevation of Factor V or VIII as predisposing to thrombosis.[4]

The platelet count for sheep ranged from 260,000–740,000 per cmm while the human blood control ranged from 200,000–400,000 per cmm.

The platelet adhesiveness in sheep varied from 58.3 to 96.9% adhered compared to human blood platelets which varied from 18 to 60% ad-

TABLE I.—*Blood Coagulation Values of Sheep*

Tests	Ovine blood				Control (human blood)
	No. of observations	Average	Minimal-maximum values	Standard deviation	
Whole blood clotting time in glass (min.)	18	9.7	5.0–15.0	3.0	5–12
Whole blood clotting time in plastic (min.)	15	31.4	5.0–57.0	17.0	20–50
Whole blood lysis time	23	None observed in 24 hours	—	—	0–20% in 24 hours
Activated partial thromboplastin time* (% of control)	18	117.4	67.3–162.0	21.9	100
Activated partial thromboplastin time (seconds)	18	31.9	20.0–52.0	9.6	28–44
Prothrombin time* (% of control)	18	95.7	80.1–122.4	12.4	100
Prothrombin time (seconds)	18	14.7	12.5–16.5	1.2	13–15
Thrombin time* (% of control)	16	108.3	85.5–135.5	17.5	100
Factor II** (% of control)	10	21.4	10.5–40.0	10.6	60–140
Factor V** (% of control)	10	416.8	160–800	227.4	60–140
Factor VII & X** (% of control)	10	50.6	16.5–100.0	33.5	60–140
Factor VIII** (% of control)	10	809.0	440.0–1650.0	408.2	50–200
Factor IX** (% of control)	10	210.7	82.0–350.0	90.5	60–140
Factor X** (% of control)	10	31.8	13.5–72.0	20.1	60–140
Factor XI** (% of control)	10	36.5	7.5–80.0	26.4	60–140
Platelet count (per cmm.)	19	457,300	260,000–740,000	120,700	200,000–400,000
Platelet Adhesiveness (% adhered)	13	79.9	58.3–96.9	13.6	18–60
Fibrinogen level (mg./100 ml)	22	291.7	132–456	80.8	200–400
Plasminogen level (units/ml.)	10	85.6	59.0–95.0	11.9	2,000–4,000
Antiplasmin level (units/ml.)	12	158.3	120.0–200.0	20.8	80–150
Euglobulin lysis time (hours)	More than 24 hours—8 sheep 12–24 hours—5 sheep 6–12 hours—5 sheep				

* Percent Value—compared with normal human plasma; control/sample × 100 = %
** Value calculated from a dilution curve of normal human plasma
From Cajewski and Povar

hered. These results and the high platelet count most likely represent an active hemostatic system.

Plasminogen level in sheep blood ranged from 59.0 to 95.0 units per ml compared to 2,000 to 4,000 units per ml in human blood. This significantly lower plasminogen level in sheep is in line with the markedly prolonged euglobulin lysis time, to be described later, indicating a very low fibrinolytic activity.

Sheep plasminogen, like bovine plasminogen, cannot be suitably activated with streptokinase alone. It was found that the addition of a trace amount of human plasma containing a "proactivator" was sufficient to develop a measurable plasmin activity and thus allowed titration of the plasminogen level. Activation was possible only at plasma concentrations ten times as great as that for human plasma. The results could conceivably have been affected by increased antiplasmin levels. However, further dilution of the sheep plasma did not increase released plasmin activity as in the case when antiplasmin is responsible for low lytic activity. The antiplasmin level was titrated and it confirmed this conclusion. This indicates that it was the low level of plasminogen that demanded such a high concentration of plasma. It should be stressed that the activation was possible only in plasma concentrations ten times greater than in human control samples and thus the results represent only approximate values.

Euglobulin lysis time in sheep was markedly prolonged as indicated above.

Number of sheep	Lysis time
8	over 24 hours
5	12–24 hours
5	6–12 hours

These results again pointed to the low fibrinolytic activity of sheep blood.

The change of the whole blood clotting time in glass measured after a sheep received an intravenous injection of heparin in doses of 1 and 2 mg/kg of body weight is shown in Figure 1. After an injection of 1 mg/kg, clotting time returned to normal within 4½ to 5 hours, while a 2 mg/kg injection resulted in a delay of 5½ to 6 hours before normalization of clotting time. The results resemble that of man whose metabolism

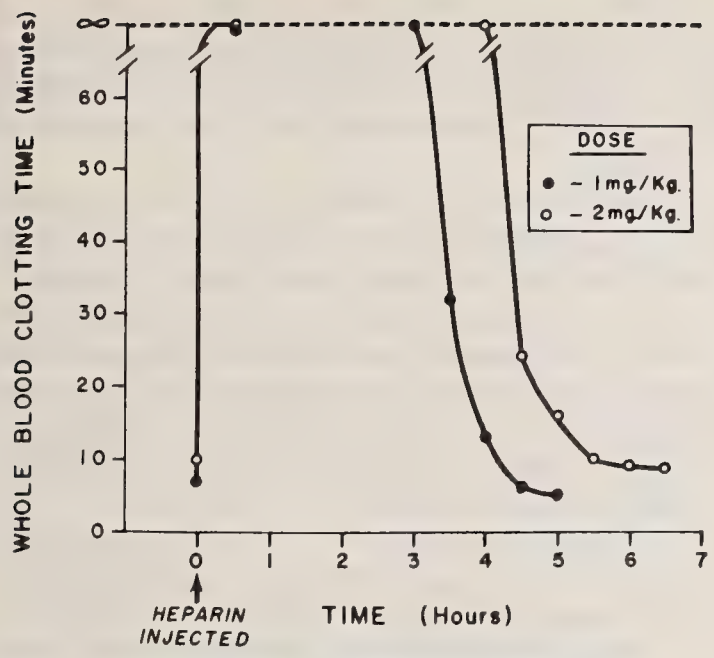


Fig. 1—Clotting time of sheep blood after a single injection of heparin.

of heparin is rapid and increases with higher blood concentrations. We have observed that injections of 10 mg/kg resulted in no apparent bleeding in the sheep and a return to normal clotting time within 24 hours.

SUMMARY

The results of this study do not confirm the opinions of Shea et al. that "rapid clotting appears characteristic of sheep and that they are relatively insensitive to conventional doses of heparin."[5] On the contrary, clotting time proved comparable to that of man. The blood of the sheep in this study was characterized by a low fibrinolytic activity, a possible increased tendency toward "coagulation," and an active hemostatic system.

REFERENCES

1. GAJEWSKI, J., and POVAR, M. L. Blood coagulation values of sheep. Am. J. Vet. Res. 32:405–409, 1971.
2. MORAG, M., and ROBERTSON SMITH, D. The day-to-day variations in certain blood values and in blood coagulation times of healthy ewes. Rehuah Vet. 28:74–78, 1971.
3. DIDISHEIM, P., HATTOVI, K., and LEWIS, J. H. Hematologic and coagulation studies in various animal species. J. Lab. & Clin. Med. 53:866–875, 1959.
4. SHERRY, S. Thrombosis (Editorial). Circulation 40:755–756, 1969.
5. SHEA, M. A., INDEGLIA, R. A., and BERNSTEIN, E. F. Hematologic observation during perfusions in sheep. In *Organ Perfusion and Preservation.* Edited by J. C. Norman. Appleton-Century-Crofts, New York, 1968, 899–909.

DISCUSSION

QUESTIONER: Why did you use a dilution factor of 1 to 200 in your factor rate dilutions? Secondly, why didn't you use a pool of sheep plasma instead of a human standard?

DR. POVAR: At the beginning of the study, Dr. Gazewski had set up a procedure for running these tests. The tests were run over a period of a month or more, and the funding ran out after that. No further tests nor work could be done on this. We're presenting this hoping to stimulate other people to carry on. There's a tremendous amount that isn't known about the procedure. I'm reporting what was done, and it is not in any way a comprehensive study.

QUESTIONER: I was just wondering why. In all the other tests, you used a dilution factor of 1 to 10 for your factor assays, but when you did factor 8 assay you used a dilution factor of 1 to 200.

DR. POVAR: I can't answer that question. I don't know why that was done.

W. J. DODDS, New York State Department of Health, Albany, N.Y.: I just have a small comment. What you're trying to tell us is that you feel the coagulation system or the hemostatic system of sheep is much more active than that of man. The point is that this is the case with most of the domestic animals whose coagulation has been studied. The system is more active. If, for example, you say that the dog has up to 10 times the factor rate as man, then a dog with mild hemophilia and a 20% factor rate would be normal because he would have 200% relative to people. However, these dogs have hemophilia and bleed. Therefore, you shouldn't use the term hypercoagulable with regard to factor 5 and factor 8 levels because they're perfectly normal in sheep.

DR. POVAR: No, we carefully worded that to suggest a possible tendency towards hypercoagulability. None of our results in the artificial

lung program indicate any tendency towards hypercoagulability. But because of the human information, Dr. Gazewski felt that a statement like that should be included.

QUESTIONER: I'm from the Naval Hospital at Bethesda. I have a question regarding your heparinization of sheep. Have you encountered GI hemorrhage when you're heparinizing for the membrane lung studies?

DR. POVAR: We've had absolutely no hemorrhaging whatsoever, much to our surprise. This is especially the case when very large doses were used which we found later were not necessary. The 2 mg. per kilogram was more than adequate and the 10 mg. per kilogram was just run as an experiment. In most of these studies the animals are not permitted to survive. They're on the artificial lung machine anywhere from six to twenty-four hours and then sacrificed under anesthesia. So from that standpoint, there is no answer. However, over a period of two years we have permitted about ten animals to recover and they've been absolutely uneventful.

QUESTIONER: You say 2 mg. per kilogram as a loading dose or as a maintenance dose?

DR. POVAR: That was a loading dose.

QUESTIONER: And this is for any type of membrane lung?

DR. POVAR: For any membrane lung that we have used, yes.

QUESTIONER: Another question. Have you been able to obtain sheep that are completely free of intestinal parasitic disease?

DR. POVAR: In the report that will be given tomorrow, we discuss this a little bit. We brought in sheep to the University of Rhode Island which had a farm that we could use. The sheep were conditioned for a period of several months, during which time their parasite load was reduced to practically nil by normal detection tests. They were checked repeatedly thereafter, and the parasite load was considered negligible.

QUESTIONER: In terms of your comment about hypercoagulability, I'd also like to point out that perhaps these high levels of factor 8 and 5 are probably counteracted by the low levels of prothrombin or factor 2. These are the same order of magnitude levels that I obtained at Washington State University some years ago on sheep. You might say from that standpoint that they're hypo.

DR. POVAR: It seems to work out that they are essentially normal. We just use the expression "tendency toward."

CHAIRMAN SEEGERS: Let's put it this way. I'm normal, but every other human being isn't. Sheep heparin is one of the weakest heparins that can be isolated or has ever been isolated. I'd like to start a discussion on hyper- and hypocoagulability of the blood. I don't get very much of anything out of that except a big wallop. What impresses me is that I isolate the prothrombin complex, add it two times, three times; even twenty times higher than normal; then I ask does that give a hypercoagulability in your test tube? No. It begins to inhibit as soon as you have more than is normally there. It simply inhibits. Yet all this talk that goes around, though perfectly adorable and wonderfully confusing, irritates me when people say that the pill raises the clotting factor by 10%. Therefore, watch out. Be very, very careful. Then the Food and Drug Administration says, "We can't tolerate that." Oh for hell's bells, for a few molecules extra they want to get the world excited. I don't believe it. I really don't.

EXPERIMENTAL APPROACHES TO THE STUDY OF VITAMIN K

Walter H. Seegers*

This is a commentary on animal experiments related to vitamin K. The rich literature on this subject began to appear in 1929 with a description of dietary experiments in which chickens were used as test animals to see whether they could live without receiving cholesterol. They developed a bleeding tendency which was found to be due to a dietary deficiency of a vitamin not previously recognized. Without vitamin K, the plasma prothrombin concentration diminished and the blood coagulation time was prolonged. Even before vitamin K was discovered, cattle had been found to develop fatal hemorrhages when fed spoiled sweet clover hay. The toxic hay was later deliberately produced; the toxin was isolated (Dicumarol), synthesized, and marketed as an anticoagulant which functioned as an antagonist to vitamin K. Rabbits were used as test animals, and many were found to be resistant to the toxin. Dogs developed vitamin K deficiency after surgical modifications which diverted bile via the urine, but the dog was mainly useful for studies on Dicumarol-vitamin K antagonism. With the successful isolation of plasma prothrombin, it was possible to build antibodies to the protein, and by using the fluorescent antibody technique demonstrate interesting aspects of prothrombin synthesis in the liver. With rats that were receiving Dicumarol, it was possible to make observations on stress. Various stressors, including some of a psychological nature, triggered internal hemorrhages that were fatal.

INTRODUCTION**

The new and revised edition of *Experiments on Animals* by Paget appeared in 1903.[1] Its mission was to point out the value of advances in biology made by experiments which required animals. I find it interesting that Lord Lister, who wrote the introduction, was the one who emphasized that surfaces are important for promoting the coagulation of blood. Today it is easy to recognize the importance of his observations and additionally to outline some of the main enzyme reactions which bring about the sol-gel transformation. In order to appreciate work on vitamin K, it is helpful to indicate briefly the nature of the chemistry of blood coagulation which is understood to date (Figure 1).

Three basic reactions occur in the given order:

(1) Formation of autoprothrombin C (Factor X_a)

(2) Formation of thrombin

(3) Formation of fibrin

Thrombin is required for the formation of fibrin and the limited proteolysis by this enzyme is quite specific, exact, unique and rather special. The formation of thrombin requires only autoprothrombin C, but the enzyme needs to have accessory substances to determine its specificity. By itself, it degrades prothrombin randomly. However, with plasma Ac-globulin, the right kind of phospholipid, and calcium ions, thrombin is formed specifically. The formation of autoprothrombin C is a more general phenomenon. During clotting there is only fractional utilization, and much remains in serum. Several substances, and combinations of substances, accelerate autoprothrombin C formation. Among these substances are tissue extracts, which have a high degree of species specificity.

It is important for our discussion to appreciate that the two main enzyme precursors, namely, autoprothrombin III (Factor X) and prothrombin, depend upon vitamin K for their synthesis in the liver. In the circulating blood, the two enzymes are bound together in the prothrombin complex.

Avian blood became a favorite fluid for investigation because it can be drawn into paraffin-lined glass vessels and, with good luck, will re-

* Department of Physiology, Thrombosis Specialized Center of Research, Wayne State University School of Medicine, Detroit, Michigan.

** This work was supported by research grant HE–03424–15 from the Heart and Lung Institute, National Institutes of Health, U. S. Public Health Service.

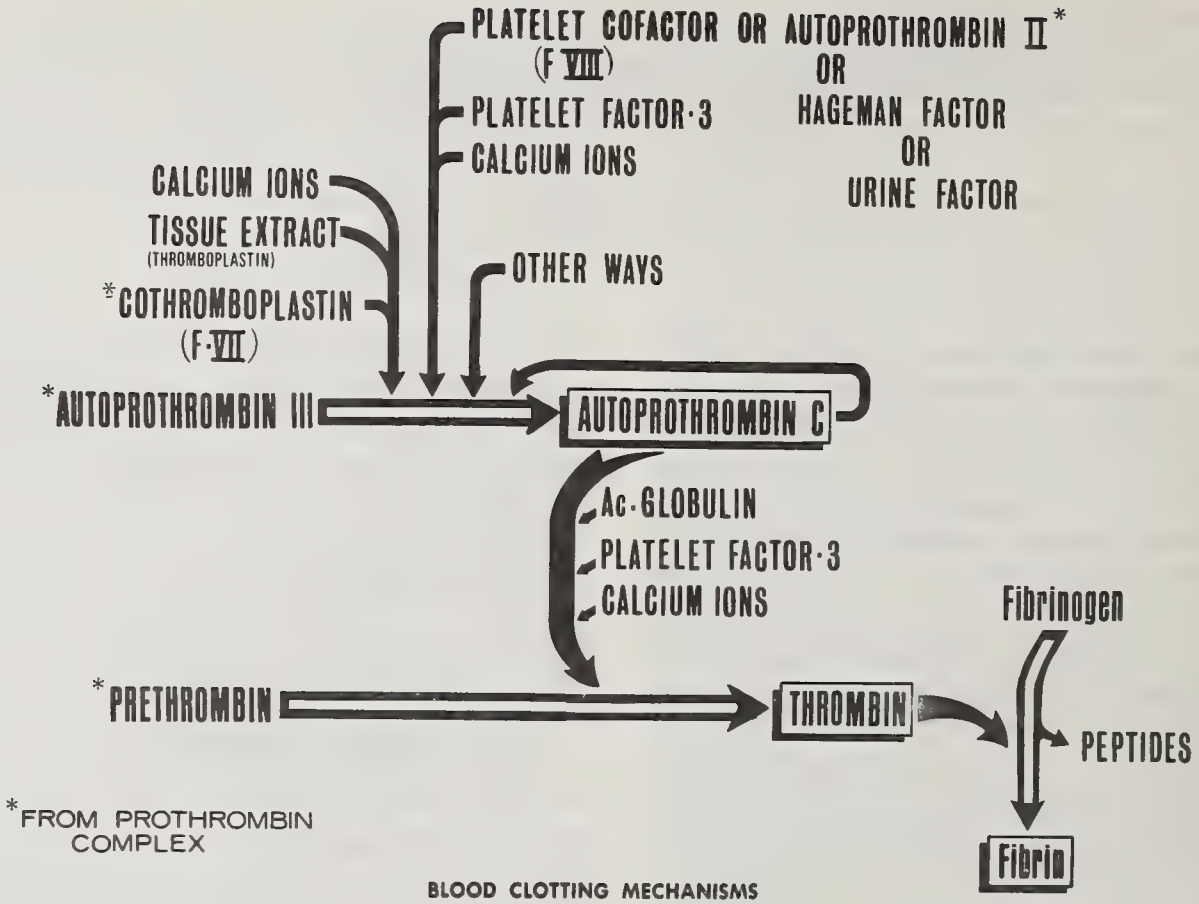


FIGURE 1.—The clotting of blood consists of three basic chemical reactions:

1) The formation of autoprothrombin C ($F\text{-}X_a$, thrombokinase, Stuart factor)
2) The formation of thrombin
3) The formation of fibrin

Thrombin alone, as a proteolytic enzyme, produces fibrin. Autoprothrombin C, another proteolytic enzyme, forms thrombin from its precursor. This function of autoprothrombin C is accelerated in a rather specific manner by plasma Ac-globulin, platelet factor 3, and calcium ions. The formation of autoprothrombin C can occur spontaneously, but is accelerated in various ways. Acceleration occurs with calcium ions, tissue thromboplastin and cothromboplastin (F–VII). The latter group of accelerators leads to complete consumption of autoprothrombin III. Incomplete autoprothrombin III utilization and less intensive acceleration of autoprothrombin C formation is obtained with calcium ions and platelet factor 3 alone or with platelet factor 3, calcium ions, plus platelet cofactor (F–VIII). In like manner, Hageman protein ($F\text{–}XII_a$) serves as a platelet cofactor. Autoprothrombin II (F–IX) is another platelet cofactor. It functions after being derived from the prothrombin complex. There are other ways to form autoprothrombin C as, for example, with a procoagulant from urine. The supplementation of platelet factor 3 activity by any one of the platelet cofactors neither requires nor excludes a preferred sequence in which the platelet cofactor activity functions. Possibly the order is Hageman protein, platelet cofactor, and lastly platelet cofactor II. Hageman protein is not necessary.

main fluid and thus be available for studies. In that way, it was possible to work in a manner which was impossible with mammalian blood, because the latter has a tendency to clot even if cold conditions are maintained.

When Heinrik Dam discovered vitamin K in 1929, he was studying the nutritional requirements of chickens.[2] He did not have blood coagulation in his plan, but wanted to see whether this species could live on a cholesterol-low diet. They developed a bleeding tendency, which proved to be a dietary deficiency not previously

Die Hühnchen erhielten mit einer Pipette so viel vom cholesterinfreien Lebertranpräparat, daß dies im Laufe eines Monats einer Menge von 15 g Lebertran entsprach, falls kein A-Stoff bei der Behandlung verloren gegangen war. Dies entspricht gut 1,5 % des Futters. Im ganzen erhielten zehn Tiere dieses Futter. Die Tiere wollten nicht gut gedeihen. Nach etwa 3 Wochen zeigten sie verschiedene innere Blutungen, und sie starben ungefähr 1 Monat alt. Die Blutungen wurden teils im Verdauungskanal, teils subkutan und intramuskulär an verschiedenen Stellen gefunden. Einige hatten zugleich Ätzwunden im Kropf, andere hatten Wasseransammlungen in der Bauchhöhle und in den Brustmuskeln. Die meisten Tiere hatten mehr oder weniger krumme Beinknochen und waren zugleich blutarm[1]. Nachdem die Blutungen entstanden waren, erhielten drei Tiere täglich 10 Tropfen Citronensaft, aber es trat keine Änderung im Zustande ein. Die Blutungen blieben auch nicht aus bei zwei Tieren, welche von Anfang an Citronensaft (steigend von 5 bis 15 Tropfen im Laufe von einem Monat) erhielten. Ein Erstatten der Salzmischungen mit dem Gemisch, welches *Hart, Halpin* und *Steenbock*[2], sowie *Hart, Steenbock*

The animals did not thrive well. After 3 weeks they developed internal bleeding at various sights and died at about an age of one month. The bleedings were partly in the intestinal tract, some were subcutaneous, and at various places intramuscular. Several also had ulcerative wounds in the gizzard, others accumulated fluid in the abdominal cavity and in the breast muscles. Most of the animals had more or less bowed leg bones and were also anemic. After the hemorrhagic condition developed, three animals were given 10 drops of lemon juice daily, but there was no change in the condition. The hemorrhages also appeared in two animals that received lemon juice at the outset (increments from 5 to 15 drops in a period of a month).

FIGURE 2.—Facsimile and translation of Dam's recorded observations on bleeding in chickens due to dietary deficiency. Later called vitamin K deficiency.

recognized (Figure 2). He isolated the active principle and called it vitamin K. This stimulated studies in blood coagulation as well as work on the importance of quinones. A few years later, I was a graduate student at the State University of Iowa where I was studying Biochemistry and Physiology (1931–1934). I can remember details about my living at the Alpha Chi Sigma house. At this professional fraternity, chemists were educating themselves along the lines of what was considered clean and respectable chemistry for them. Physiology and Biochemistry were tolerated with the attitude that "rat feeders" would not completely degrade the standards of the institution. In this fraternity of brilliant scholars, the chickens of Heinrik Dam would certainly have had nothing to crow about. I notice with great satisfaction that the hard preliminary work in the field of blood clotting enzymology is now finished. Next, we can expect the clean chemistry. I mention these prejudices because I believe that narrow views of scientists are a part of the perspective on animal experimentation as well as other scientific work.

Observing diseases in animals has been a dominant way to learn about the coagulation of blood. Dicumarol and its congeners were found in that way. I presume it is well-known that this drug is used extensively to deal with thrombosis problems and owes its effectiveness to reducing the concentration of the plasma prothrombin complex. It antagonizes the function of vitamin K. Before the latter was discovered, the effects of Dicumarol were observed in cattle that were by chance fed spoiled sweet clover hay.[3,4] This forage crop was introduced in Ontario, Canada. It was alarming to see herds of cattle die of internal hemorrhages. In North Dakota, U.S.A., the same was happening and the defect in blood coagulation was identified as a prothrombin deficiency. In Wisconsin, dairy cattle also developed the deficiency. A method was found to produce the toxin.[5] It was isolated, synthesized, and introduced for clinical use.

During the isolation work, rabbits were used as test animals. Some of these were found to be resistant to the drug on a genetic basis. Later, such resistance was also found in rats. Once many of us hoped that anticoagulants would serve the purpose of exterminating the rat as a pest. Certainly many have gone to sleep in that way, but now it has been discovered that large numbers of brown "house" rats *(Rattus norvegicus)* and black "ship" rats *(Rattus rattus)* can eat the anticoagulant and continue to enjoy it. New immune strains have developed. Such animals are, of course, especially useful for studying the synthesis of the prothrombin complex. It has been found that some human beings are also resistant to the drug.[6] In fact, the report on this subject documents the first instance of genetically determined drug resistance in man.

In man, hemophilia was popularly associated

only with the male. It is a sex-linked, inherited irregularity. Observing hemophilia in dogs served as another approach to research. The colony of animals developed and housed by Kenneth M. Brinkhous and his associates[7] at the University of North Carolina is a record in high adventure in nursing ailing animals and finding out the nature of their difficulty. Through breeding experiments, he and his associates were able to produce hemophilia A like disease in female dogs. Dogs with Factor IX deficiency are also known. A more detailed summary of this work is given by Brinkhous.[8]

I want to refer to another nuance in this field of research. It was convenient to take blood from human patients or hemophilic dogs, add plasma fractions, and see which ones restored the clotting time to normal. This provided an assay for Factor VIII activity. This might have been an exclusive way for testing blood fractions, but we managed to devise an equally reliable test based on the use of purified blood components. This was called the platelet cofactor assay (Figure 3). We did not need animals or hemophiliacs to do our work. Now I encountered the reverse of the "rat feeder attitude." This time I was dealing with nice reagents in test tubes and did not need animals for assays. However, when our results were not liked, the suggestion came through quite strongly: "But you have not actually performed tests on deficiency plasma; therefore, you cannot be certain that you are measuring Factor VIII activity." I presume this "scientific strategy" helps create the kind of confusion a competent trial lawyer can deliberately feature. Actually, we worked with the deficient plasmas as well as the purified coagulation components. One of the hallmarks of good experimental work is exploration by utilizing many avenues of approach. It has unfortunately been difficult to find funds for research with animals having hereditary disorders.

Pigs with von Willebrand's disease were first discovered at the University of Missouri and described by Hogan, Muhrer, and Bogart.[9] Such animals were found to have a long bleeding time and low levels of Factor VIII. Following infusion of plasma or serum, the Factor VIII levels were found to be considerably greater than could be accounted for on the basis of the amount of Factor VIII infused.[10] Perhaps there is a von Willebrand stimulating factor. Some years after the initial studies were recorded, Albert Hogan, who was chairman of the department in which the work was done, explained that no one was studying these pigs and he thought it might be best to destroy them. When my opinion was solicited, I urged him not to do it. Now, new information is being produced and animals from this hereditary line are also being studied at the Mayo Clinic.[11] With advancing age, a pig keeps on growing and proves to be a large animal for experimental work.

Returning to studies on vitamin K, I select one of our experiments with the dog as an example (Figure 4). The prothrombin and autoprothrombin III (factor X) concentration was measured while Dicumarol was administered in large amounts. The concentration of the two

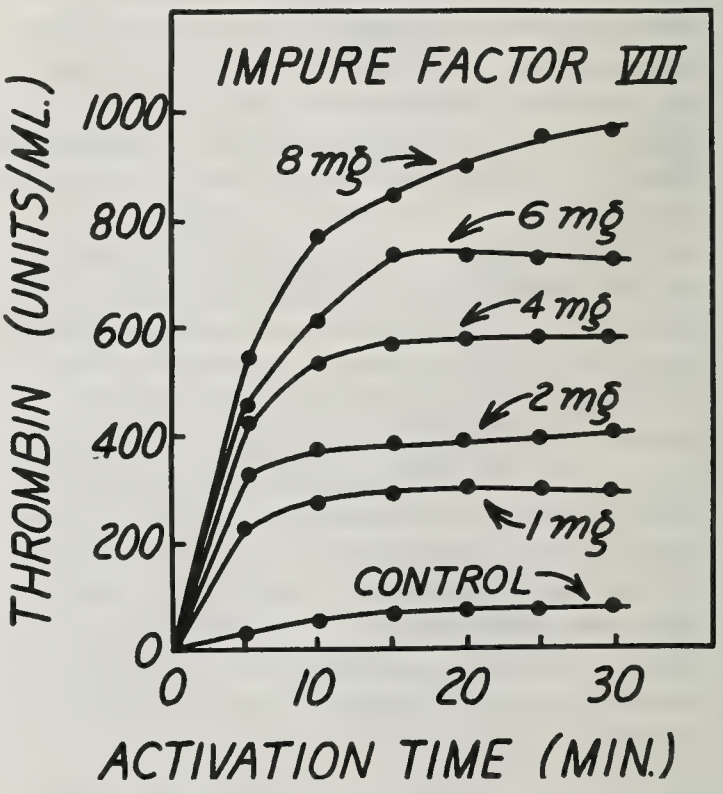


FIGURE 3.—Assay for Factor VIII. Thrombin formed from purified prothrombin complex in the presence of purified Ac-globulin (Factor V), platelet factor 3, 0.01 M calcium chloride, and variable amounts of a platelet cofactor (Factor VIII) concentrate. Note that the thrombin yield depended upon the quantity of platelet cofactor added to the reaction mixture. At pH 7.2 and 37° C.

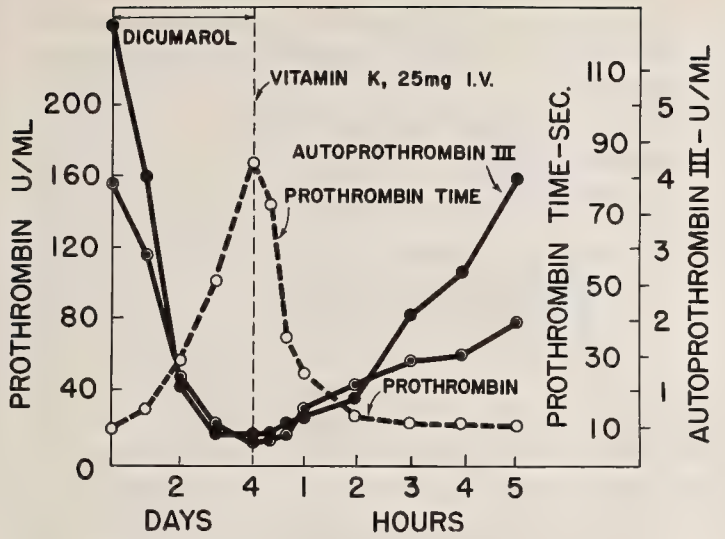


FIGURE 4.—Prothrombin time, two-stage prothrombin assay, and autoprothrombin III concentration in the plasma of an 18 kg. dog. On successive days 100, 75, and 50 mg Dicumarol were given. Then, the effect was reversed by the intravenous infusion of vitamin K_1.

proenzymes diminished at the same rate over a period of four days. The antagonist completely suppressed the synthesis of the vitamin K dependent protein(s). When vitamin K was then given intravenously to overcome the inhibition, an increase in the concentration of plasma prothrombin was already noticed within one hour. The upward trend continued during the five hours of observation. By using fluorescent antibodies for prothrombin, it was demonstrated that fluorescence could be seen on liver parenchymal cells within 30 to 60 minutes after the administration of vitamin K.[12] It is really marvelous to observe the control of special protein synthesis in this way. It has been calculated that 1.27×10^{15} prothrombin molecules were synthesized per minute after the vitamin K was given. It has a molecular weight of about 69,000 and is only a part of the prothrombin complex. It is hard to comprehend how so many amino acids find their proper place so rapidly.

Another special feature of this experiment illustrates that the prothrombin time is no indicator of prothrombin or autoprothrombin III concentration during the period when vitamin K initiates the synthesis. No one has produced data to explain the peculiar response of the prothrombin time. In perfusion experiments, livers have been taken from animals previously treated with Dicumarol and a shortening of the prothrombin time has been observed. Various contradictory interpretations have been given to the results.

The experiment of 1968, illustrated by means of Figure 4, was possible because Dicumarol and vitamin K were available in bottles. Many years before that, namely, in 1938, I participated in an experiment that took 750 days instead of five.[13] By surgical procedures on a dog, we arranged to have the bile drain through the kidney and out with the urine. As a consequence, the fat-soluble vitamin K was not well absorbed and a prothrombin deficiency developed (Figure 5). When bile was fed, the deficiency slowly subsided and developed once more when the bile was not fed. This experiment alerted investigators to the possibility that vitamin K might be useful for human beings afflicted with obstructive jaundice.

An important biological phenomenon for animals and man is STRESS. The great contribution of Hans Selye was his demonstration that stress produces changes in many body organs and tissues of rats and that these changes are frequently adverse. A problem with the use of drugs, which inhibit blood coagulation or affect platelets, and problems with various conditions (e.g., hemophilia) which have the same result, is that hemorrhage can result in an unpredictable manner. Louis B. Jaques and associates [14-16] have shown, with the use of rats, that this is due to accompanying stress. This provides one of the simplest ways of demonstrating and studying stress in animals. When an anticoagulant was administered and the animals were stressed, most of them developed symptoms of internal hemorrhage in four or five days, and this was measured by the incidence of such hemorrhage, degree of hemorrhage or mortality from hemorrhage. In this way, stress was demonstrated to have occurred in rats exposed to confinement, electroshock, psychological conditioning procedures, many drugs (including anesthetics), injection procedures, etc. Even handling of older rats to receive a subcutaneous injection twice a day proved to be a stress. This is an example of how animal studies can throw light on a serious clinical problem which is resistant to analysis clinically.

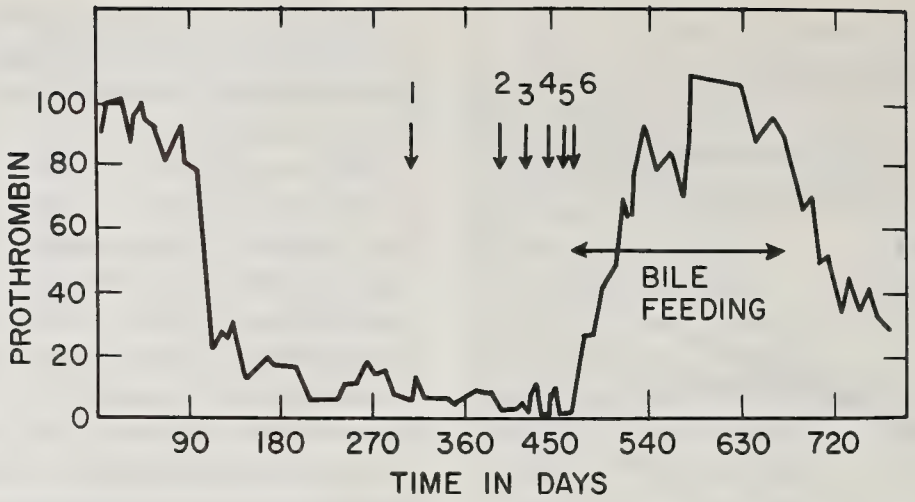


FIGURE 5.—Prothrombin concentration in bile drained dog. Two-stage prothrombin analysis. Arrows indicate events by number: (1) bleeding from operative stitch abscess, 210 ml transfusion; (2) vaginal bleeding; (3) bleeding from venipuncture site, 130 ml transfusion; (4) bleeding from venipuncture site, 110 ml transfusion; (5) some bleeding again, 160 ml transfusion; and (6) once more, 75 ml transfusion. With bile feeding prothrombin concentration increased.[12]

These glimpses from a really rich literature must suffice. It would have been interesting to give attention to snake venoms and the importance of those creatures for this work. I add the interesting fact that the hibernation of the ground squirrel is a special condition for studying thrombosis.[17] With inactivity and sluggish circulation, conditions are created which are precisely conducive to problems with thrombosis. Nothing of the sort happens, apparently because the blood platelet count drops to low levels.

For me, it is self-evident that work with animals that have genetically determined deficiencies ought to be conducted on a much more extensive scale. Comparative work is likely to be fruitful. Surely, the advances made in the biochemistry of blood coagulation will now lead to extensive developments in the comparative aspects, and I have in mind all those animals with an adequate plasma supply. The study of hemostasis and thrombosis has yielded theoretical and practical results, and I presume anyone interested would find it a most rewarding investment of his time.

REFERENCES

1. PAGET, S. *Experiments on Animals.* G. P. Putnam's Sons, New York, 1903.
2. DAM, H. Cholesterinstoffwechsel in Huhnereiern und Huhnchen. Biochem. Ztschr., 215:475–492, 1929.
3. RODERICK, L. M. The pathology of sweet clover disease in cattle. J. Amer. Vet. Med. Ass., 74:314–326, 1929.
4. SCHOFIELD, F. W. A brief account of a disease in cattle simulating hemorrhagic septicaemia due to feeding sweet clover. Canad. Vet. Rec., 3:74, 1922.
5. LINK, K. P. The discovery of Dicumarol and its sequels. Circulation, 19:97–107, 1959.
6. O'REILLY, R. A., AGGELER, P. M., HOAG, M. S., LEONG, L. S., and KROPATKIN, M. L. Hereditary transmission of exceptional resistance to coumarin anticoagulant drugs. New Eng. J. Med., 271:809–815, 1964.
7. BRINKHOUS, K. M., and GRAHAM, J. B. Hemophilia in the female dog. Science, 111:723–724, 1950.
8. BRINKHOUS, K. M. Summary of contribution of hemophilic dogs to knowledge and human welfare, Research Animals in Medicine, National Heart and Lung Institute, NIH, HEW, 1972.
9. HOGAN, A. G., MUHRER, M. E., and BOGART, R. A hemophilia-like disease in swine. Proc. Soc. Exp. Biol. Med., 48:217–219, 1941.
10. MUHRER, M. E., LECHLER, E., CORNELL, C. N., and KIRKLAND, J. L. Antihemophilic factor levels in bleeder swine following infusions of plasma and serum. Amer. J. Physiol., 208:508–510, 1965.
11. CHAN, J. Y. S., OWEN, C. A., JR., BOWIE, E. J. W., DIDISHEIM, P., THOMPSON, J. H., JR., MUHRER, M. E., and ZOLLMAN, P. E. Von Willebrand disease "stimulating factor" in porcine plasma. Amer. J. Physiol., 214:1219–1224, 1968.

12. Anderson, G. F. and Barnhart, M. I. Prothrombin synthesis in the dog. Amer. J. Physiol., 206:929–938, 1964.
13. Smith, H. P., Warner, E. D., Brinkhous, K. M., and Seegers, W. H. Bleeding tendency and prothrombin deficiency in biliary fistula dogs: Effect of feeding bile and vitamin K. J. Exp. Med., 67:911–920, 1938.
14. Jaques, L. B. Stress and multiple factor etiology of bleeding. Ann. N. Y. Acad. Sci., 115:78–96, 1964.
15. Jaques, L. B. *Anticoagulant Therapy: Pharmacological Principles.* Charles C. Thomas, Springfield, Ill., 1965.
16. Jaques, L. B. Effects of hormones and drugs on hemostasis. Drugs in Relation to Blood Coagulation, Hemostasis and Thrombosis. III International Pharmacological Congress, São Paulo, Brazil, July 24–30, 1966. Vol. 26, pp. 25–55, Pergamon Press, Oxford and New York.
17. Lechler, E., and Penick, G. D. Blood clotting defect in hibernating ground squirrels *(Citellus tridecemlineatus).* Amer. J. Physiol., 205:985–988,

DISCUSSION

P. L. Blackshear, University of Minnesota, Minneapolis: Dr. Seegers, could you say some more about stress, please? Does stress only reduce the tendency to coagulate or does it increase the tendency for clotting?

Dr. Seegers: The stress which was produced in these animals produces hemorrhages. The rats were given Dicumarol in rather high concentration, and then stress was introduced. The number of rats that died were then counted. On autopsy they were found to have widespread internal hemorrhages of all kinds. These were even produced if, for example, a workman who didn't usually work there went in to replace one that was customarily there. This was sufficient to disturb the animals and made enough of them bleed to death on that psychological stress alone. Sham injections of saline alone produced the same kind of response. In other words, these animals were depending on three main things for their hemostasis: first the blood vessel wall, then the platelets and the coagulability of the blood. If the coagulability of the blood was reduced to a very low level, one could then bring on hemorrhage by interferring with any of the other two. One way of doing this is with stress.

Morris L. Povar, Brown University, Providence, R.I.: Dr. Leo Vroman from the Veteran's Hospital in New York reports that when he surveys a number of different animals and surveys dogs under varying degrees of stress, that the tendency to clot in his dogs covers the whole range of the many species that he investigates. He didn't give the dogs any drugs. He simply treated them poorly, got them excited, or babied them to make them feel secure. Have you observed such a range in any single species and then compared it with a number of different species?

Dr. Seegers: I must add I'm sorry if I left the impression that I had been doing any work on the stress situation. I am delighted to hear you mention the work of Dr. Vroman. I read his works too.

SUMMARY OF CONTRIBUTION OF HEMOPHILIC DOGS TO KNOWLEDGE AND HUMAN WELFARE

Kenneth M. Brinkhous*

A canine animal model identical with an inherited sex-linked human disease, hemophilia, has been of greatest value in elucidating the basis of the disease and in developing a rational therapeutic regimen.[1] This model has contributed in two major ways: (a) by a study of the disease in the animals themselves, and (b) by supplying a ready source of blood plasma for biochemical and physiological studies of a plasma procoagulant, the antihemophilic factor (AHF, Factor VIII). In the first category, experiments, with the deficient animals included studies on inheritance, methodology for diagnosis, pathophysiology, and therapy. In regard to inheritance, it was demonstrated that classic hemophilia could be produced in females[2] and that with these females all major genetic crosses of recessive sex-linked Mendelian inheritance were for the first time completed.[3] The low level of AHF in female carriers was verified and the diagnostic value of this test in identifying this class of females, even at birth, was demonstrated.[4] Genetic linkage studies with subluxation of the carpus[5] and pseudohermaphroditism[6] and chromosomal DNA replication[7] were undertaken. By interbreeding with a strain of hemophilia B dogs, the loci of the two hemophilia genes were shown to be widely separated, and a new strain of animals with double hemophilia or hemophilia AB was established.[8,9] In methodologic and pathophysiologic studies two bioassay methods were developed,[10,11] and on one of these, the partial thromboplastin time, the diagnostic schema almost universally adopted in clinical laboratories is based.[11,12] The anticephalin hypothesis was tested.[13] The short half-life and biphasic disappearance curves of transfused AHF[12,14,15] became the basis of scheduled plasma or concentrate therapy to maintain hemostasis. The rapid loss of AHF during clotting was established[1,16] and the relation of feedback mechanisms due to formation of thrombin[17,18] and plasmin (or fibrinolysin)[19] was shown, and the ineffectiveness of subcutaneous and intramuscular routes for treatment was demonstrated.[20]

Through organ perfusion and organ transplantation studies, the role of the spleen as a site of storage of AHF[21–24] and the curative role of the transplanted normal liver were established.[25] The role of the kidney was explored.[26] The resistance of AHF levels to total body irradiation was demonstrated.[27] The finding that hemophilic dogs were resistant to diffuse intravascular coagulation (DIC)[28,29] was the forerunner of the paradox of treating DIC with anticoagulants, heparin and Dicumarol.

By having a reliable AHF deficient plasma constantly available, the biochemical characterization of AHF was started,[14,30–34] and intermediate and high-potency concentrates (glycine-precipitated) for therapy of hemophilia were developed.[35,36] AHF was shown to be a cryoprotein, a forerunner of cryoprecipitate therapy.[37,38] It was shown that an antibody to AHF characterized by a high degree of species specificity could be produced.[39]

* Department of Pathology, University of North Carolina, School of Medicine, Chapel Hill, North Carolina.

REFERENCES

1. Graham, J. B., Buckwalter, J. A., Hartley, L. J., and Brinkhous, K. M. Canine hemophilia: Observations on the course, the clotting anomaly, and the effect of blood transfusions. J. Exp. Med. 90:97, 1949.
2. Brinkhous, K. M. and Graham, J. B. Hemophilia in the female dog. Science 111:723, 1950.
3. Brinkhous, K. M. Hemophilia. Bull. N. Y. Acad. Sci. 3:325, 1954.
4. Parks, B. J., Brinkhous, K. M., Harris, P. F., and Penick, G. D. Laboratory detection of female carriers of canine hemophilia. Thromb. Diath. Haemorrh. 12:368, 1964.

5. Pick, J. R., Goyer, R. A., Graham, J. B., and Renwick, J. H. Subluxation of the carpus in dogs, an X chromosomal defect closely linked with the locus for hemophilia A. Lab. Invest. 17:243, 1967.
6. Brown, R. C., Swanton, M. C., and Brinkhous, K. M. Canine hemophilia and male pseudohermaphroditism: Cytogenetic studies. Lab. Invest. 12:961, 1963.
7. Brown, R. C., Castle, W. L. K., Huffines, W. D., and Graham, J. B. Pattern of DNA replication in chromosomes of the dog. Cytogenetics 5:206, 1966.
8. Davis, P. D. Canine double heterozygotes for hemophilia A and B. Fed. Proc. 27:627, 1968.
9. Brinkhous, K. M., Davis, P. D., and McLester, W. D. Combined hemophilia A-B in dogs: Affected males and carrier females from crossbreeding. XIII International Congress of Hematology, Munich. Abstract Volume, p. 221, 1970.
10. Graham, J. B., Collins, D. L., Godwin, I. D., and Brinkhous, K. M. Assay of plasma antihemophilic activity in normal, heterozygous (hemophilia) and prothrombinopenic dogs. Proc. Soc. Exp. Biol. Med. 77:294, 1951.
11. Langdell, R. D., Wagner, R. H., and Brinkhous, K. M. Effect of antihemophilic factor on one-stage clotting tests. J. Lab. Clin. Med. 41:637, 1953.
12. Brinkhous, K. M., Penick, G. D., Langdell, R. D., Wagner, R. H., and Graham, J. B. Physiologic basis of transfusion therapy in hemophilia. Arch. Path. 61:6, 1956.
13. Graham, J. B. and Barrow, E. M. The pathogenesis of hemophilia. An experimental analysis of the anticephalin hypothesis in hemophilic dogs. J. Exp. Med. 106:273, 1957.
14. Brinkhous, K. M., Langdell, R. D., Penick, G. D., Graham, J. B., and Wagner, R. H. Newer approaches to the study of hemophilia and hemophilioid states. J. A. M. A. 154:481, 1954.
15. Langdell, R. D., Wagner, R. H., and Brinkhous, K. M. Antihemophilic factor (AHF) levels following transfusions of blood, plasma and plasma fractions. Proc. Soc. Exp. Biol. Med. 88:212, 1955.
16. Graham, J. B., Penick, G. D., and Brinkhous, K. M. Utilization of the antihemophilic factor during clotting of canine blood and plasma. Amer. J. Physiol. 164:710, 1951.
17. Brinkhous, K. M. and Wagner, R. H. Antihemophilic factor (AHF): Some aspects of its biochemistry. In: *Blood Clotting Factors.* Proceedings of the Fourth International Congress of Biochemistry, Vienna, 1958. Edited by E. Deutsch, Pergamon Press, New York, Vol. X, p. 1, 1959.
18. Penick, G. D. Some factors that influence utilization of antihemophilic activity during clotting. Proc. Soc. Exp. Biol. Med. 96:277, 1957.
19. Wagner, R. H., Brinkhous, K. M., and Penick, G. D. Antihemophilic factor: Some problems in its study. In: *Hemophilia and Other Hemorrhagic States.* Edited by K. M. Brinkhous, University of North Carolina Press, Chapel Hill, p. 3, 1959.
20. Wagner, R. H., Langdell, R. D., Richardson, B. A., Farrell, R. A., and Brinkhous, K. M. Antihemophilic factor (AHF): Plasma levels after administration of AHF preparations to hemophilic dogs. Proc. Soc. Exp. Biol. Med. 96:152, 1957.
21. Gross, J. D., Hartmann, R. C., Graham, J. B., and Taylor, C. B. Splenectomy in hemophilia. Bull. Johns Hopkins Hosp. 100:223, 1957.
22. Weaver, R. A., Price, R. E., and Langdell, R. D. Antihemophilic factor in cross-circulated normal and hemophilic dogs. Amer. J. Physiol. 206:335, 1964.
23. Webster, W. P., Reddick, R. L., Roberts, H. R., and Penick, G. D. Release of factor VIII (antihaemophilic factor) from perfused organs and tissues. Nature 213:1146, 1967.
24. Webster, W. P., Penick, G. D., Peacock, E. E., and Brinkhous, K. M. Allotransplantation of spleen in hemophilia. N. C. Med. J. 28:505, 1967.
25. Webster, W. P., Zukoski, C. F., Hutchin, P., Reddick, R. L., Mandel, S. R., and Penick, G. D. Plasma factor VIII synthesis and control as revealed by canine organ transplantation. Amer. J. Physiol. 220:1147, 1970.
26. Barrow, E. M., and Graham, J. B. Antihemophilic factor activity isolated from kidneys of normal and hemophilic dogs. Amer. J. Physiol. 220:1020, 1971.
27. Penick, G. D., Cronkite, E. P., Godwin, I. D., and Brinkhous, K. M. Plasma antihemophilic activity following total body irradiation. Proc. Soc. Exp. Biol. Med. 78:732, 1951.
28. Brinkhous, K. M., and Penick, G. D. Some systemic alterations in clotting factors following local tissue injury. In: *Thrombosis and Embolism.* Proceedings of the First International Conference, Basel, 1954. Edited by T. Koller and W. R. Merz, Benno Schwabe and Company, Basel, p. 428, 1955.
29. Penick, G. D., Roberts, H. R., Webster, W. P., and Brinkhous, K. M. Hemorrhagic states secondary to intravascular clotting. Arch. Path. 66:708, 1958.
30. Wagner, R. H., Richardson, B. A., and Brinkhous, K. M. A study of the separation of fibrinogen and antihemophilic factor (AHF) in canine, porcine, and human plasmas. Thromb. Diath. Haemorrh. 1:1, 1957.
31. Barrow, E. M., Amos, S. M., Heindel, C., and Graham, J. B. Separation of the antihemophilic factor (F. VIII) from fibrinogen with thrombin and manganese chloride. Proc. Soc. Exp. Biol. Med. 121:1001, 1966.
32. Barrow, E. M., Amos, S., and Graham, J. B. Certain biochemical properties of human AHF (factor VIII) separated from fibrinogen with manganous chloride and thrombin. J. Lab. Clin. Med. 68:803, 1966.

33. WAGNER, R. H., and THELIN, G. M. Antihemophilic factor: Current status of purification, chemical and physical characterization. In: *Hemophilia and Hemophilioid Diseases.* Edited by K. M. Brinkhous, University of North Carolina Press, Chapel Hill, p. 3, 1957.
34. THELIN, G. M., and WAGNER, R. H. Sedimentation of plasma antihemophilic factor. Arch. Biochem. Biophys. 95:70, 1961.
35. WEBSTER, W. P., ROBERTS, H. R., THELIN, G. M., WAGNER, R. H., and BRINKHOUS, K. M. Clinical use of a new glycine-precipitated antihemophilic fraction. Amer. J. Med. Sci. 250:643, 1965.
36. BRINKHOUS, K. M., SHANBROM, E., ROBERTS, H. R., WEBSTER, W. P., FEKETE, L., and WAGNER, R. H. A new high-potency glycine-precipitated antihemophilic factor (AHF) concentrate. J. A. M. A. 205:613, 1968.
37. BRINKHOUS, K. M. Plasma antihemophilic factor, biological and clinical aspects. Sang 25:738, 1954.
38. HERSHGOLD, E. J., POOL, J. G., and PAPPENHAGEN, A. R. The potent antihemophilic globulin concentrate derived from a cold insoluble fraction of human plasma: Characterization and further data on preparation and clinical trial. J. Lab. Clin. Med. 67:23, 1966.
39. MCLESTER, W. D., and WAGNER, R. H. Antibody to antihemophilic factor and its lack of reaction with hemophilic plasma. Amer. J. Physiol. 208:499, 1965.

A SURVEY OF BLOOD GROUPS IN SEVERAL SPECIES OF LARGE ANIMALS USED IN MEDICAL RESEARCH

Clyde J. Stormont*

The first systematic studies of blood groups in large species of domestic animals were begun on cattle in the late 1930's in the laboratory of M. R. Irwin at the University of Wisconsin. The appropriate serological approach, which had long been overlooked, was the preparation of blood typing reagents from isoimmune antisera and the use of hemolytic tests rather than agglutination tests, the latter being clearly contraindicated for the red cells of such species as cattle. This approach paid off. Already by 1941 some 30 isoantigenic determinants on cattle red cells were known.[1a,b] That number was somewhat unprecedented when we consider that only about eight markers (A_1 A_2, M, N, P, Rh_o and rh′) were then recognized on human red cells.

In the course of exploring blood groups in domestic animals numerous firsts have come to light. Of particular significance among those firsts was the discovery, by R. D. Owen, of erythrocyte mosaicism or blood group chimerism in dizygotic cattle twins and higher zygotic multiples.[2a,b,c] His observations stimulated much of the work on immunological tolerance and are basic to theories on that subject.[3] I, therefore, take this opportunity to set forth a number of additional firsts which have influenced our thinking about blood groups.

INTRODUCTION

Most of the investigations of animal blood groups are contemporary, that is, from about 1940 onwards. The same is true of human blood groups with the exception of the earlier studies on the ABO groups, M-N types and P types. This is not to say that there was no work or little work on animal blood groups prior to 1940. On the contrary, there were many studies but, for the most part, they were transient surveys concerned with a search for naturally occurring isoagglutinins that might detect in animals a system of blood groups akin to the ABO groups of man. Those earlier studies have been concisely reviewed by Wiener.[4]

Today there is an extensive literature about blood groups in cattle, horses, pigs and sheep but much less so for dogs. There are several reasons for the less extensive literature on dog blood groups, and I would like to touch upon those reasons at this point. The lack of literature on dog blood groups is not because of a lack of interest in dog blood groups. On the contrary, there have been numerous attempts to produce antibodies that might detect individual differences in the antigenic structure of dog erythrocytes, as evidenced, for example, by the efforts of L. E. Young and colleagues. (See review by Swisher et al.[5]) In spite of the efforts, only 8 blood factors have been identified to date. Three (C, F and G) are of such high frequency (incidence greater than 0.98) that they are of little interest in routine typing, and apparently none of the laboratories presently involved in dog blood typing has available more than 4 or 5 different typing fluids.[5,6]

Another problem left unmentioned in reports on blood groups in dogs is that dog red cells are difficult to work with because of the existence on those cells of an autolytic factor which renders them vulnerable to hemolysis when washed and suspended in virtually any neutral serum. This autolytic factor, which seems to be an attribute of the red cells of all dogs, is much like that encountered by us in blood typing studies on the red cells of certain Arabian horses.[7]

One might ask, what kind of information about animal blood groups would those who use the larger species of domestic animals for physiological studies, experimental surgery and organ transplantation be particularly interested in? If the answer relates mainly to transfusion reactions and the possibility of using the antigenic markers as indicators of histocompatibility, I could complete my assignment here in a few paragraphs.

Irrespective of incompatibilities in blood types, reactions on initial transfusions are rarely seen in cattle, dogs, pigs and sheep.

* Serology Laboratory, Department of Veterinary Microbiology, University of California, Davis, California.

When, however, initial transfusions are followed by one or more additional transfusions at intervals of several days or weeks, the reactions can be severe.[1a,5] Such reactions are almost always due to isoimmune antibodies engendered as a result of the initial transfusions or arising as a consequence of vaccination with homologous tissue vaccines. Failure to elicit reactions on initial transfusions cannot be attributed to the absence of naturally occurring isoantibodies. On the contrary, most J-negative cattle, R-negative sheep and A-negative pigs manifest the equivalent isoantibodies. And I understand (personal communication, Dr. J. W. Templeton, this Conference) some A-negative dogs manifest anti-A. But, in contrast with anti-A and anti-B of man, the titers of these natural antibodies are generally low, and most of the antibodies act much better as hemolysins than they do as agglutinins, thereby indicating that embolism due to *in vitro* clumping of red cells is less likely to occur. Moreover, in the case of anti-J, anti-R and anti-A there is usually abundant soluble blood group substances in the plasma and tissues of those animals possessing the equivalent antigens and these soluble substances bind with the antibodies and prevent them from reaching the red cells.

There is, as far as I am aware, no blood group locus in cattle, dogs, pigs, sheep and horses which functions also as a major histocompatibility locus, like for example, the H-2 loci of mice. Consequently, none of the antigenic markers on the red cells of those species is useful as an indicator of histocompatibility.

The problems associated with transfusion reactions and the rejection of tissue grafts and organ transplants can be circumvented by using cattle twins. Most dizygotic cattle twins, and higher zygotic multiples, come to share each other's hematopoietic tissues as a result of chorionic vascular anastomosis.[2] They are tolerant not only to each others blood but also to grafts of each other's skin.[8] Therefore, they should also be tolerant to transplants of each other's organs. Monozygotic twins are, of course, just as useful, if not more so, for such experiments, but they occur much less frequently than chimeric cattle twins.

Blood typing can be used to diagnose chimeric and monozygotic twins. Likewise, when twins reject allografts of each other's skin it is clear that they cannot be chimeric or monozygotic. In view of the ease of diagnosis, either by blood typing or skin grafting, it comes as a surprise that such twins are not in greater demand for experimental surgery and organ transplantation.

Chimeric twins also occur in sheep but their rarity precludes their extensive use in medical research.[9,10]

SOME FIRSTS IN ANIMAL BLOOD TYPING

In addition to the discovery, by blood typing, of chimeric twins and higher chimeric multiples in cattle and sheep, there are other firsts for animal blood typing which are often overlooked in treatises on blood groups in man.

One of those firsts relates to the initial discovery of isoantigenic determinants. Although Landsteiner is often credited with the discovery of the ABO blood groups of man in 1900, and did in fact observe isoagglutination when he mixed the serum of certain persons with the red cells of others, the first description of the existance of isoantigenic determinants in any species was set forth by Ehrlich and Morgenroth.[11a] They injected individual goats intraperitoneally with rather large quantities of pooled, laked blood of other goats and then sampled the injected goats at regular intervals for the appearance of isolysins and autolysins. Although no autolysins were detected, isolysins did appear, some arising as early as two or three days and others only after several weeks. Each of the isoimmune antisera was of a different specificity, as ascertained by testing them with a panel of goat and sheep red cells. The authors mentioned four different isolysins (A, B, C and D) and, in a subsequent note,[11b] Ehrlich pointed out that altogether 13 specificities were defined. He concluded, therefore, that red blood cells possess a large number of receptors (i.e., isoantigenic determinants) which probably belong to hundreds of different types, but it was not until some 40 years later that the real significance of his remarks could be appreciated.

That 1900 paper by Ehrlich and Morgenroth was of particular significance in two ways. It

pointed out the most effective method of developing antibodies of diverse specificity for use in exploring blood groups, namely, isoimmunization. And it indicated the use of hemolytic tests where agglutination tests are clearly contraindicated, namely, those species like cattle, goats and sheep whose red cells are not prone to agglutinate even when saturated with multiple doses of blood typing antibodies. Unfortunately, neither of those observations became generally appreciated until the 1940s.

Another first which bears mentioning here was the discovery of what are nowadays referred to as "inclusion" groups, such as the inclusion group *BGK* of cattle where blood factor K makes it appearance only in the presence of both B and G, although the latter two occur alone and together in the absence of K.[12] Many inclusion groups of the order *BGK* have since been discovered, including *cef* of the Rh-Hr blood groups in man. They are of unusual interest in immunogenetics because they defy interpretations based on closely linked genes.[13] On the other hand, they connote cross or overlapping reactions among the isoantigenic determinants on red cells, and are readily interpretable in those terms. Blood factors B, G and K of cattle segregate as the elements of a series of related determinants defined by multiple allelic genes B^{BGK}, B^{BG}, B^{B}, B^{G} and *b*, the latter coding for the absence of B, G and K. Discovery of inclusion groups of the order *BGK* were of particular importance for two reasons: (1) they brought to light the existence of determinants that are capable of binding antibodies of more than one specificity, and (2) they have helped considerably in the elucidation of such complex blood group systems as B of cattle.[14]

Although it is generally assumed that all antigenic determinants recognized on cell membranes are intrinsic properties of those membranes, there are some notable exceptions, including the Lewis determinants on human red cells.[15] These exceptional determinants are soluble substances produced in the tissues, secreted into the blood plasma and acquired by the red cells on contact with them in blood plasma. The first example of a red cell determinant of that order was J of cattle, as shown by both *in vivo* and *in vitro* experiments,[16] and this observation was followed by studies[17] which showed that the R and O properties of sheep red cells are also acquired from blood plasma. Aside from ascertaining the origin of certain antigenic determinants on red cells, these observations were of particular significance in accounting for the reasons why chimeric twins in cattle, sheep and man may differ in their J types, R-O types and Lewis types, respectively. It is clear from such studies that the tissues which give rise to these soluble substances are not exchanged by the twins *in utero*.

It is axiomatic in immunogenetics that any antigenic determinant, whether membrane bound or not, is inherited as a dominant in contrast with its absence. There are a few exceptions to that rule, the first bonafide example being the O determinant of sheep which is inherited as a recessive to R.[18]

It is also axiomatic that genes controlling antigenic determinants are expressed in any genotype. Here, again, the first bonafide exceptions were provided by studies on animal blood groups. Utilizing anti-sheep O provided by the author, Rendel et al.[17] observed that some sheep, rather rare in occurrence,[19] failed to manifest R and O substances on their red cells and in their plasma. They designated this third group in the R-O system as group i and established that a gene, designated *I*, at a second locus, is necessary for the expression of groups R and O. Thus, sheep of genotypes *RRII*, *RRIi*, *RrII* and *RrIi* express R in their plasma and on their red cells, and sheep of genotypes *rrII* and *rrIi* express O in their plasma and on their red cells, whereas sheep of genotypes *RRii*, *Rrii* and *rrii* do not do so. This kind of gene interaction is referred to in genetics as epistasis, and the example involving groups R, O and i of the R-O system of sheep constituted the first example of epistasis involving antigenic determinants. Since then there have been others, for example, the A-O groups of pigs[20] and the ABO groups of man.[21] The existence of epistasis is particularly important when interpreting the results of blood typing tests in connection with problems of questionable parentage. It is possible, as an example, for sheep of blood group O (genotype *rrII*) to produce nothing but group R offspring

(genotype *RrIi*) in matings to group i sheep of genotype *RRii*.

That the R-O system of sheep has some physiological significance is indicated by the restriction of an alkaline phosphatase isozyme (zone B) to sheep of group O.[22,23] But, in that connection, the more recent developments relating the blood group locus M of sheep to high (HK) and low (LK) red cell potassium types are of more than usual interest because they establish that antigenic determinants on cell membranes are involved in the transport of ions across those membranes.

In 1966, Rasmusen and Hall[24] made the observation that the genes which control the M blood groups of sheep[25] also control potassium levels. They observed that all HK sheep are homozygous for the alleles which code for the M determinants whereas LK sheep are either heterozygous or homozygous for the allele(s) which codes for the absence of the M determinants.

The next obvious thing to do was to attempt to produce isolysins specific for the product(s) of the non-M allele(s). Both Rasmusen[26] in the United States and Ellory and Tucker[27] in Britain were successful. At first those antibodies were designated anti-m but the name was wisely changed to anti-L, the reactions being limited, as they were, to the red cells of LK sheep. Now, in that same report, Ellory and Tucker recorded a most significant observation. They observed that when the red cells of LK sheep, either homozygous or heterozygous for the L determinant, are sensitized with anti-L there is a four-to-five-fold stimulation of potassium transport. In other words, they accomplished *in vitro* the conversion of LK red cells to type HK by loading the L receptor sites with anti-L. This observation was soon confirmed by Lauf et al.[28] as reviewed by Tucker.[10]

The significance played by antigenic determinants, or the molecules with which they are associated, in regulating such things as the potassium level of red cells is yet to be appreciated. However, if the variation in antigenic determinants is largely associated with the immune response and thereby has evolved along with the evolution of that response, it seems likely that the effect of the O determinant of sheep on the appearance of an alkaline phosphatase isozyme or the L determinant on the level of red cell potassium may be purely coincidental and thereby of no unique selective value.

SEROLOGY AND GENETICS OF ANIMAL BLOOD GROUPS

In spite of the claim[29] that the great bulk of blood group knowledge has been gathered from the results of simple (saline) agglutination tests, it may come as a surprise for some to learn that the foregoing firsts in animal blood typing were all brought to light by the use of hemolytic rather than agglutination tests. Except for the studies on pig blood groups and those of dogs (Table I), the bulk of blood group knowledge, at least insofar as our larger species of laboratory animals may be concerned, has been gathered from the results of hemolytic tests.

While simple in principle, none of the procedures used in blood typing is without its risks. Blood typing tests are extremely delicate, and require constant surveillance. Hemolytic tests, when properly performed, produce the most sharply defined and easily readable reactions. Moreover, the amount of antibody required to produce hemolysis is usually far less than that required to produce agglutination. For example, it has been estimated that two or three molecules of 19S (IgM) antibody is sufficient to produce hemolysis of a sheep red cell,[49] but this may be a special case since it relates to the Forssman determinant.

Complement is necessary for hemolysis and the key to the development of effective hemolytic tests is the use of an appropriate complement. This is almost never fresh neutral serum of the species under study. For example, fresh neutral cattle serum is useless for cattle blood typing. It will produce stickiness and thereby agglutination of sensitized cattle red cells but such agglutination reactions are poorly defined with the average reagent used in cattle blood typing. Moreover, they are readily distinguishable from regular agglutination because the clumps disaggregate as the complement becomes inactive.

TABLE I.—*The approximate number of blood factors, recommended typing procedures, and the phenotypic systems of blood groups identified in cattle, dogs, horses, pigs and sheep*

Species	Approximate no. of blood factors	Typing procedures in order of preference	Recognized phenotypic systems of blood groups; minimum no. of alleles shown within parentheses	References
			12 systems	
			A (10),** B (500+), C (70+), F-V (5),	14, 16, 30, 31,
			J or J-Oc (4+), L (2), M (3), N (2),	32, 33, 34, 35,
Cattle	80	Hemolysis only	S or S-U (8), Z (3), R'-S' (3),** and T' (2)	48, 50, 51, 53.
		Agglutination	The 9 canine blood factors (A_1, A_2, B, C, D, E,	
		Hemolysis	F, G, and Tr) are yet to be classified with	
Dogs	9	(A_1 only)	respect to phenotypic systems.	5, 6, 36.
			8 systems	
		Hemolysis	A (5), C (2), D (6), K (2), P (3), Q (6),**	37, 38, 39,
Horses	20	Agglutination	T (2), and U (2)	40, 41, 48.
			15 systems	
			A-O (A locus 2; S locus 2), B (2),** C (2),	
			D (2),** E (13),** F (3),** G (2),** H (6),	
		Agglutination*	I (2),** J (3), K (5), L (6),** M (9),	42, 43, 44,
Pigs	60	Hemolysis	N (3),** and O (2).**	48, 52.
		Hemolysis	7 systems	10, 17, 18, 23,
		Agglutination	A (2), B (60+), C (3), D (2), M (4),**	25, 26, 27, 45,
Sheep	50	(D only)	R-O (R locus 2; I locus 2) and X-Z (2).**	46, 47, 48, 54.

* Some of the agglutinins act as so-called incomplete agglutinins and require the use of anti-globulin to bring about agglutination. Some of the others produce agglutination when the red cells are suspended in dextran solutions. The majority, however, act as saline agglutinins.

** Closed systems. That is, there is no phenotype which is non-reactive with all of the reagents used in typing the particular system. All others are open systems. That is, there is one phenotype in the particular system which is characterized by the absence of reactions with all reagents used in typing that system.

Generally, fresh rabbit serum, now commercially available (Pel Freez Biologicals, Rogers, Arkansas), is the complement of choice for cattle blood typing. It is also the complement of choice in cytotoxicity tests (e.g., the human leukocyte A system). There are, however, certain cattle reagents which produce better hemolysis with guinea pig complement but such reagents are exceptional.

Fresh rabbit serum has one major disadvantage when employed in tests on the red cells of Forssman-positive species (dogs, goats, horses, pigs and sheep). The reason for this is that rabbits, being Forssman-negative, develop, naturally, Forssman antibodies which, in conjunction with rabbit complement, will lyse the red cells of such species as dogs, horses, pigs and sheep. But such antibodies, and all other interferring natural antibodies, can be absorbed from fresh serum without affecting the complement if the absorptions are performed at 0–4°C using red cells of the species under study, a methodology described by Ehrlich and Morgenroth[11c] in 1899.

With few exceptions, as already noted, each of the numerous blood factors is inherited as a dominant in contrast with its absence. But not all blood factors are inherited independently of one another as already indicated for blood factors which belong to inclusion groups. All blood factors fall naturally into one or another phenotypic system of blood groups (Table I) and much of the effort in blood typing studies is devoted to the analysis of such systems.

Perhaps the simplest systems of all are the two-allele, closed systems, and there are numerous systems of that order, particularly in pigs (Table I). They do, however, on further study, have a tendency to expand into multiple allelic systems, a notable example being the F-V system of cattle.[30] As a model of two-allele, closed systems, consider the X-Z system of sheep with two alleles, X and X^z, and three phenotypes X XZ and Z, conveniently written as X/X, X/Z and Z/Z to indicate the contribution of each parent to the phenotypes of their offspring. Such systems are referred to as "closed" systems because each phenotype is represented by at least one blood factor. This is in contrast to the two-allele, open systems which involve only a single blood factor, one phenotype being represented by the presence of the particular blood factor and the other by its absence. There are numerous examples of such systems.

At the opposite extreme are the multi-allelic systems like A, B, C and S of cattle, A, D and Q of horses, E, H and M of pigs, and B of sheep. Some of these multi-allelic systems are open

systems and others are closed. The most complex system of this order so far recognized in any species is the B system of cattle, undoubtedly followed by its less extensively explored homologue, the B system of sheep. Both are open systems in spite of the large number of blood factors which belong to those systems.

The blood factors B, G and K which belong to the inclusion group *BGK* are members of the B system of cattle and were the starting point[14] of the genetic analysis of that system, which presently involves approximately one-half of the 80 or so blood factors recognized in cattle (Table I). In the B system, as in other multi-allelic systems, blood factors segregate as the properties of units referred to as phenogroups (e.g., the phenogroups I_2, Y_1, Y_2, E'_1, I', BI_1, O_1I', $E'_1I'_1$ GO_1Y_2, $GY_2E'_1$, $Y_1D'I'$, BO_1Y_2D', $BGKE'_2O'$, . . . etc., through phenogroups of the order $BGKO_2Y_1A'B'E'_3G'KO'Y'$ in the B system of cattle). Over 500 phenogroups of the order shown have been recognized to date in studies of the B system of cattle. They differ markedly in their breed distributions. Thus, the phenogroup formula $BGKO_2Y_1A'B'E'_3G'K'O'Y'/O_1T_1E'_3F'K'$ is typical of Jerseys but is never seen in breeds such as Herefords, Angus, Holstein-Friesians and so on. Consequently, it is relatively easy for the experienced operator to identify the breed of cattle from which a blood sample is drawn merely by examining the B phenogroups. The diploid combinations of the 500+ B phenogroups of cattle give rise to thousands of possible blood types and, when all those blood types are considered in conjunction with those generated by alleles at the remaining 11 loci, the overall number of different blood types is far greater than the number of cattle that have populated this planet.[30] There is also, as indicated by the figures in Table I, an astounding number of possible blood types in pigs. At the opposite extreme is the picture in dogs. Ignoring the difference between A_1 and A_2, and assuming that each of the canine blood factors may be inherited independently of one another, the number of possible blood types in dogs is presently only about 2^8. But considering the fact that few, if any, laboratories type for the high frequency factors F and G, the realized variation is much less.

As might be expected in such complex systems as B of cattle, there is occasional recombination of the blood factors indicative of crossing over at the locus or chromosomal region which controls the B phenogroups.[50] Recently, Bouw and Fiorentini[51] have constructed a map of the B region of the chromosome. This map, based on a study of 57 instructive recombinants in 30,000 offspring, indicates some 16 points of possible crossing over. Similarly, Baker[52] has recently constructed a tentative map of the region which controls the E system of pigs. Thus, for the first time in animals larger than a mouse we are beginning to learn something about the fine structure of the loci controlling blood groups.

GENE OR CHROMOSOMAL HOMOLOGIES

It is not, of course, possible to hybridize such species as cattle and sheep and, thereby, study the segregation of blood factors in F_2 hybrids or back cross hybrids. Consequently, the only indicators of homologous blood group systems in the species considered here are cross reacting antibodies. Thus, anti-J and anti-sheep O of cattle are excellent antibodies in this context. Not only do they define individual differences in the J or J-Oc system of cattle[53] but they are also effective reagents in defining the R-O groups of sheep[17] and the A-O groups of pigs.[20] Moreover, anti-J of cattle cross reacts with A of man,[16] thereby establishing homologies between the ABO system of man, J of cattle, R-O of sheep and A-O of pigs. The report by Bowdler et al.[6] would indicate that the Tr factor of dogs also belongs to this system.

Many of the reagents which define individual differences in the B system of cattle also define individual differences in the B system of sheep, and the converse is also true.[54] Similar homologies have been established between the C system of cattle and the C system of sheep, and the S system of cattle and the M system of sheep.

When, for example, the red cells of domestic sheep and other species of the families Bovidae and Cervidae are injected into rabbits they give rise to some antibodies which react specifically with the A determinants on cattle red cells, thereby indicating that the A system of

cattle also has its homologue in those species. But the gene or genes determining the A factor in such species as domestic sheep appears to be at or near fixation, as is true in most instances for the gene or genes controlling Forssman blood factors.[55] Undoubtedly, many additional homologies will be brought to light on further comparisons, and all will be useful in constructing phylogenetic trees based on biochemical and antigenic markers.

SUMMARY

Cross matching blood before initial transfusions in cattle, dogs, pigs and sheep is of little practical importance because of the rarity of transfusion reactions. Where multiple transfuions are given, severe and sometimes fatal reactions may be expected as a result of isoimmune antibodies engendered by the recipients. Transfusion reactions are also expected when recipients have been immunized with homologous tissue vaccines.

None of the blood group loci in the species under consideration functions as a major histocompatibility locus. Consequently, pairing of donors and recipients for allografts on the basis of their blood groups serves no worthwhile purpose. On the other hand, chimeric cattle twins should be ideal for such experiments. Not only do they share each other's hematopoietic tissues and therefore can be cross-transfused at will, but they are also tolerant to homografts of each other's tissues. It is recommended that such twins, which are easily diagnosed by blood typing or by exchanging skin grafts, be used for organ transplantation experiments.

There are numerous firsts, such as the discovery of blood group chimerism in dizygotic twins and higher zygotic multiples, which have been brought to light as a consequence of studies on blood groups of such species as cattle and sheep. Some of those firsts are outlined here.

All of these break-throughs have come about by the use of hemolytic tests. The importance of utilizing hemolytic typing procedures cannot be over-emphasized.

The antigenic factors on red cells which define individual differences fall naturally into one or another phenotypic system of blood groups. The number of recognized systems varies between species, ranging from 7 in sheep to 15 in pigs. Cattle, with 12 systems, exhibit the most complex systems of all and thereby the most phenotypic variability in blood types of any species so far studied.

There are numerous blood group homologies between cattle and sheep, as would be expected of species that belong to the same family. There are also broad-range or heterogenetic homologies which include the ABO system of man, the A-O system of pigs, the J-Oc system of cattle, the R-O system of sheep, and the Tr system of dogs.

REFERENCES

1a. Ferguson, L. C. Heritable antigens in cattle blood. J. Immunol. 40:213–242, 1941.

b. Ferguson, L. C., Stormont, C., and Irwin, M. R. On additional antigens in cattle blood. J. Immunol. 44:147–164, 1942.

2a. Owen, R. D. Immunogenetic consequences of vascular anastomosis between bovine twins. Science 102:400–401, 1945.

b. Owen, R. D. Erythrocyte mosaics among bovine twins and quadruplets. Genetics 31:277, 1946.

c. Owen, R. D., Davis, H. P., and Morgan, R. F. Quintuplet calves and erythrocyte mosaicism. J. Hered. 37:290–297, 1946.

3. Burnett, M. *Self and Not-Self.* Cambridge University Press, London, 1969.

4. Wiener, A. S. *Blood Groups and Transfusion,* Third Ed. Charles C. Thomas, Springfield, Ill., 1945.

5. Swisher, S. N., Young, L. E., and Trabold, N. *In vitro* and *in vivo* studies of the behavior of canine erythrocyte-isoantibody systems. Ann. N. Y. Acad. Sci. 97:15–25, 1962.

6. Bowdler, A. J., Bull, R. W., Slating, R., and Swisher, S. N. Tr: a canine red cell antigen related to the A antigen of human red cells. Vox Sang. 20:542–544, 1971.

7. Suzuki, Y. and Stormont, C. Genetic control of an *in vitro* autolytic factor in horse red cells, pp. 525–526 in *XIIth European Conference on Animal Blood Groups and Biochemical Polymorphism.* Ed. by Kovács, G. and Papp, M. Akadémiai Kiadó, Budapest, 1972.

8. Anderson, D., Billingham, R. E., Lampkin, G. H., and Medawar, P. B. The use of skin grafting to distinguish between monogygotic and dizygotic twins in cattle. Heredity 5:379–397, 1951.

9. Stormont, C., Weir, W. C., and Lane, L. An example of erythrocyte mosaicism in a pair of sheep twins. Science 118:195–196, 1953.

10. Tucker, E. M. Genetic variation in the sheep red blood cell. Biol. Rev. 46:341–386, 1971.
11. Ehrlich, P. *Studies in Immunity*, 2nd Ed., collected and translated by Bolduan, C. John Wiley and Sons, N. Y., 1910.
    a. Chapter III. Studies on hemolysins. Third communication. Ehrlich, P. and Morgenroth, J., 1900.
    b. Chapter XXXIII. The receptor apparatus of the red blood cells. Ehrlich, P., 1901.
    c. Chapter I. Contributions to the theory of lysin action. Ehrlich, P. and Morgenroth, J., 1899.
12. Stormont, C., Irwin, M. R., and Owen, R. D. A probable allelic series of genes affecting cellular antigens in cattle. Genetics 30:25–26, 1945.
13. Stormont, C. Linked genes, pseudoalleles and blood groups. Amer. Naturalist 89:105–116, 1955.
14. Stormont, C., Owen, R. D., and Irwin, M. R. The B and C systems of bovine blood groups. Genetics 36:134–161, 1951.
15. Sneath, J. S. and Sneath, P. H. A. Adsorption of blood-group substances on to red cells. Brit. Med. J. 15:154–157, 1959.
16. Stormont, C. Acquisition of the J substance by the bovine erythrocyte. Proc. Nat. Acad. Sci. U. S. 35:232–237, 1949.
17. Rendel, J., Neimann-Sorensen, A., and Irwin, M. R. Evidence for epistatic action of genes for antigenic substances in sheep. Genetics 39:396–408, 1954.
18. Stormont, C. An example of a recessive blood group in sheep. Genetics 37:577–578, 1951.
19. Stormont, C., Suzuki, Y., Bradford, G. E., and King, P. A survey of hemoglobins, transferrins and certain red cell antigens in nine breeds of sheep. Genetics 60:363–371, 1968.
20. Rasmusen, B. A. Gene interaction and the A-O blood-group system in pigs. Genetics 50:191–198, 1964.
21. Watkins, W. M. and Morgan, W. T. J. Possible genetical pathways for the biosynthesis of blood group mucopolysaccharides. Vox Sang. 4:97–114, 1959.
22. Rendel, J. and Stormont, C. Variants of ovine alkaline serum phosphatases and their association with the R-O blood groups. Soc. Exp. Biol. & Med. 115:853–856, 1964.
23. Rasmusen, B. A. Inheritance of R-O-i blood groups and alkaline phosphatase polymorphism in sheep. Genetics 51:767–770, 1965.
24. Rasmusen, B. A. and Hall, J. G. Association between potassium concentration and serological type of sheep red blood cells. Science 151:1551–1552, 1966.
25. Rasmusen, B. A., Stormont, C., and Suzuki, Y. Blood groups in sheep. III. The A, C, D and M systems. Genetics 45:1595–1603, 1960.
26. Rasmusen, B. A. A blood group antibody which reacts exclusively with LK sheep red blood cells. Genetics 61:s49, 1969.
27. Ellory, J. C. and Tucker, E. M. Stimulation of the potassium transport system in low potassium type sheep red cells by a specific antigen antibody reaction. Nature 222:477–478, 1969.
28. Lauf, P. K., Rasmusen, B. A., Hoffman, P. G., Dunham, P. B., Cook, P., Parmelee, M. L., and Tosteson, D. C. Stimulation of active potassium transport in LK sheep red cells by blood group-L-antiserum. J. Membrane Biol. 3:1–13, 1970.
29. Race, R. R. and Sanger, R. *Blood Groups in Man*, 4th Ed. F. A. Davis, Philadelphia, 1962.
30. Stormont, C. Current status of blood groups in cattle. Ann. N. Y. Acad. Sci. 97:251–268, 1962.
31. Stormont, C. and Cumley, R. W. Cellular antigens in cattle blood. J. Hered. 34:35–41, 1943.
32. Grosclaude, F. and Millot, P. Allèles supplementaries au locus S de groupes sanguins des bovins. Ann. Biol. anim. Bioch. Biophys. 3:119–124, 1963.
33. Grosclaude, F. Note préliminaire sur un locus supplémentaire de groupes sanguins des bovins, le locus T′. Ann Biol. anim. Bioch. Biophys. 5:403–406, 1965.
34. Erhard, L., Schleger, W., Schroffel, J and Stukovsky, J. Subtypes in the R′/S′ cattle blood group system, pp. 143–145 in *XIth European Conference on Animal Blood Groups and Biochemical Polymorphism*. Dr. W. Junk N. V., The Hague, 1970.
35. Osterhoff, D. and Politzer, N. F^t- a new allele in the bovine FV blood group system, pp. 135–142 in *XIth European Conference on Animal Blood Groups and Biochemical Polymorphism*. Dr. W. Junk N.V., The Hague, 1970.
36. Cohen, C. and Fuller, J. L. The inheritance of blood types in the dog. J. Hered. 44:225–228, 1953.
37. Podliachouk, L. Les groupes sanguins des équidés (cheval, mulet, âne). Ann. Inst. Pasteur 95:7–22, 1958.
38. Franks, D. Horse blood groups and hemolytic disease of the newborn foal. Ann. N. Y. Acad. Sci. 97:235–250, 1962.
39. Stormont, C., Suzuki, Y., and Rhode, E. A. Serology of horse blood groups. Cornell Vet. 54:439–452, 1964.
40. Stormont, C. and Suzuki, Y. Genetic systems of blood groups in horses. Genetics 50:915–929, 1964.
41. Sandberg, K. Blood group factors and erythrocytic protein polymorphism in Swedish horses, pp. 447–452 in *XIth European Conference on Animal Blood Groups and Biochemical Polymorphism*. Dr. W. Junk N. V., The Hague, 1970.
42. Andresen, E. Blood groups in pigs. Ann. N. Y. Acad. Sci. 97:205–225, 1962.
43. Saison, R. and Ingram, D. G. A report on blood groups in pigs. Ann. N. Y. Acad. Sci. 97:226–232, 1962.

44. DINKLAGE, H. Results of the third pig blood group comparison test, pp. 359–361 in *XIth European Conference on Animal Blood Groups and Biochemical Polymorphism.* Dr. W. Junk N. V., The Hague, 1970.
45. RASMUSEN, B. A. Blood groups in sheep. I. The X-Z system. Genetics 43:814–821, 1958.
46. RASMUSEN, B. A. Blood groups in sheep. Ann. N. Y. Acad. Sci. 97:306–319, 1962.
47. SCHMID, D. O. New aspects of sheep blood groups, pp. 561–566 in *XIIth European Conference on Animal Blood Groups and Biochemical Polymorphisms.* Ed. by Kovács, G. and Papp, M. Akadémiai Kiadó, Budapest, 1972.
48. RASMUSEN, B. A. Blood group alleles in domestic animals, in *Handbook of Genetics.* Ed. by King, R. C. (In Press).
49. HUMPHREY, J. H. and DOURMASHKIN, R. R. Electron microscope studies of immune cell lysis, pp. 175–189 in *CIBA Foundation Symposium on Complement,* Ed. by Wolstenholme, G. E. W. and Knight, J. Little, Brown & Co., Boston, 1965.
50. STORMONT, C. Mammalian Immunogenetics, pp. 715–722 in *Proc. XI Int. Cong. Genetics, Vol. 3.* Ed. by Geerts, S. J., Pergamon Press, N. Y., 1965.
51. BOUW, J. and FIORENTINI, A. Structure of loci controlling complex blood group systems in cattle, pp. 109–113 in *XIth European Conference on Animal Blood Groups and Biochemical Polymorphism.* Dr. W. Junk N. V., The Hague, 1970.
52. BAKER, L. N. E red cell system in pigs: irregular genetic transmission. Vox Sang. 21:57–64, 1971.
53. SPRAGUE, L. M. On the recognition and inheritance of the soluble blood group property "Oc" of cattle. Genetics 43:906–912, 1958.
54. RASMUSEN, B. A. Blood groups in sheep. II. The B system. Genetics 45:1405–1417, 1960.
55. STORMONT, C. and SUZUKI, Y. The distribution of Forssman blood factors in individuals of various Artiodactyl species. J. Immunol. 81:276–284, 1958.

DISCUSSION

J. MOOR-JANKOWSKI, New York University School of Medicine, New York: Dr. Stormont, certainly in your work you have tried to use your antisera for testing closely related species to the species in which they are produced. Could you say a few words about this?

DR. STORMONT: Dr. Moor-Jankowski wants to know whether we use the antisera produced for blood-typing cattle for typing other closely related species. The answer is yes. Some of the antisera or cattle blood typing reagents are most effective in sheep blood typing. We have a blood group system in sheep known as the B system, which is homologous to the B system in cattle. Some of the antibodies that react in the B system in sheep, and iso-immunizations of sheep, cross-react neatly with those in the B system of cattle. The C system in sheep and cattle are homologous. The A system of man, the RO system of sheep, the J system of cattle, the AO system of pigs, and even the TR system in dogs are all related through antigen A of man. That is a long series of cross-reactions involving the A substance.

CHAPTER VI

HEMODYNAMICS

Cardiovascular Hemodynamics in Domestic Ponies

Comparative Mechanical Blood Properties

The Effects of the Catecholamines and Glucagon on the Performance of the Equine Heart

An Analysis of Left Ventricular Dimensional Changes in Conscious Animals

A Method of Study the Regulation of Coronary Flow and of Other Cardiovascular Functions in the Unanesthetized Performing Dog

Rheologic Properties of Blood Vessels

Requirements for Long-Term Cardiopulmonary Bypass Circulation: Hypotheses and Data from Research Animals

Hemodynamics in the Chimpanzee: Unanesthetized and Anesthetized

CARDIOVASCULAR HEMODYNAMICS IN DOMESTIC PONIES

James F. Amend, Harold E. Garner, John P. Rosborough and Hebbel E. Hoff*

Parameters of heart rate, systemic and pulmonary pressure and vascular resistance, cardiac output and index, stroke volume and index, and ventricular stroke work were determined in thirty-one unanesthetized, standing domestic ponies. Average heart rate for the group was 59 beats/min. Cardiac output (per kg body weight) was 113 ml per minute per kg. Average cardiac index was 5.1 L/min/sq meter. Systolic pressure averaged 144 mm Hg on the systemic side (central aorta), and 45 mm Hg in the pulmonary artery. Aortic diastolic pressure was 107 mm Hg; diastolic pressure in the pulmonary artery averaged 20 mm Hg. Systemic vascular resistance was 0.70 peripheral vascular resistance units (PRU), while pulmonary vascular resistance was 0.20 PRU. Stroke volume averaged 1.64 ml/beat/kg, and ventricular stroke work was 7.8 joules/stroke/kg heart weight. Additional indirect indicators of cardiac function were obtained from measurements of phases of ventricular systole, including left ventricular pre-ejection period (PEP), left ventricular ejection time (LVET), PEP/LVET ratio, isovolumic contraction time (ICT), LVET/ICT ratio, Q-S1, S1-S2, Q-S2, and QT. Significant fits were obtained for linear regression equations describing LVET, PEP/LVET, S1-S2, and Q-S2 as functions of heart rate. No significant fit was found for PEP, ICT, Q-S1, or LVET/ICT. QT was described by the asymptotic Bazett equation, where QT is equal to Bazett's constant (0.4 for the pony) times the square root of the R-R interval length. ETI was 0.593, about twice that determined in man. The regression equation LVET = 0.593 — 0.0036 HR shows that LVET shortens three times faster in the pony than in man for equal increments in rate. Additional observations of importance included comparison of the above parameters in a pony which was found to have coarctation of the aorta and bilateral ventricular hypertrophy, and in a group of animals which showed hemodynamic function similar to that seen in human labile hypertension.

INTRODUCTION**

Recent investigations in the development and evaluation of prosthetic devices for mechanical circulatory assistance have employed several species of medium-sized domestic animals as surgical and physiological models; most prominent among these has been the calf. The sheep, goat, and pig have also been used, but have proven less desirable. Notable for its absence from this group is the small domestic pony. The pony possesses many of the attributes which have made the calf a popular model; among these are availability, docility, and approximation of man in body mass and circulatory capacity. Additionally, however, the properly selected small pony may be used at maturity, while the calf is similar to man in size only at an early age. Such a factor is of considerable importance with regard to stamina required for surgical procedures, the fragility of blood vessels, maintenance of good health throughout a prolonged period of chronic experimentation, and the very important problem of growth, which can itself be a limiting factor in the life of an implanted device.

The pulmonary microanatomy of the horse and pony has been described as more similar to that of man than even the non-human primates;[1] one of the major problems of assisted circulation has been the disturbance of function in the pulmonary circulation. The pony is an athletic animal, in contrast to the calf, and can readily be trained to exercise. Certainly the stress of exercise can be the ultimate test of an assist device, thus the availability of an appropriate animal model may be of critical importance when such a stage of development is reached. Finally, the pony, as a non-ruminant, can be a more easily handled surgical and postoperative patient.

Unlike the calf,[2,3] the pony has not been subjected to controlled study to determine normal values for parameters of cardiac and hemodynamic function. This report describes results

* Department of Physiology, Baylor College of Medicine, Houston, Texas.

** This study was supported in part by U.S. Public Health Service Grants HE-05125, GM-38193.01, GM-44624.01, and RR-05425, and by research contracts from Chemagro Corporation, Kansas City, Missouri, and from Hoffman-LaRoche, Inc., Nutley, New Jersey.

of such a cardiovascular examination in a number of small domestic ponies, and discusses both properties of the cardiovascular system and suitability of the animal as a surgical and physiological model.

MATERIALS AND METHODS

Selection, Preconditioning, Housing, and Handling

The experimental group was composed of thirty-one ponies. Fourteen were males, thirteen were females, and four were geldings. Ages of the animals ranged from eight months to nine years, and weights from 53.6 to 161.5 kg. Animals were selected so that, in most cases, weight did not exceed 100 kg and age did not exceed three years. Docility, minimal training, and ease of handling were additional constraints. Each pony was quarantined for two weeks prior to delivery, and treated during this time to eliminate intravascular or gastrointestinal parasites. Upon arrival in the laboratory, ponies were placed in box stalls[4] and allowed twenty-four to forty-eight hours during which to accommodate to the unfamiliar surroundings.

Anesthesia and Surgery

Ponies were premedicated with acetylpromazine or Rompun (Bay Va 1470) prior to induction of anesthesia. A combination of 0.2% pentothal sodium and 5% guaiacol glyceryl ether was given intravenously until loss of equilibrium occurred. An endotracheal tube was then passed, and surgical anesthesia established with a combination of oxygen, nitrous oxide, and halothane. The left carotid artery was exposed, and two No. 14 Teflon catheters were placed in it and advanced until their tips lay in the arch of the aorta. A large bore polyvinylchloride catheter was placed in the left jugular vein. Upon completion of the surgery, ponies were given an intravenous infusion of 5% dextrose (One L) containing Butazolidin (600 mg), Liquamycin (500 mg), and sodium heparin (10,000 units). An intramuscular injection of tetanus antitoxin was also given as a precaution. The catheters were filled with heparinized saline and capped. Forty-eight hours of post-operative recovery were allowed before any measurements were taken.

Physiological Measurements

All data were obtained from unmedicated standing ponies, using only mild restraint which was provided by a small, mobile cart.[5] Parameters were recorded on magnetic tape, and displayed on a three-channel Physiograph. Heart rate was measured from the R-R interval of the electrocardiogram, which was recorded from silver-silver chloride disk electrodes in a chest-to-back configuration. Blood pressure was obtained from one of the indwelling carotid catheters, using a Statham AA (0–300 mm Hg) pressure transducer. A 125 cm No. 9 french right heart sampling catheter was passed through the jugular implant to the right ventricle and pulmonary artery. Pulmonary artery pressure was measured from the right heart catheter using a Statham BB (0–50 mm Hg) transducer. Cardiac output was determined by dye dilution, using indocyanine green dye and a Waters densitometer. Dye was injected into the pulmonary artery via the right heart catheter, and blood was withdrawn through the densitometer cuvette from one of the aortic catheters; the other was used to simultaneously monitor blood pressure.

Cardiac output, stroke volume, vascular resistance, and stroke work, together with related indices, were calculated by an IBM 7094 computer, using modifications of equations described by Liu.[6] Simultaneous tracings of electrocardiogram, phonocardiogram, and the aortic pressure wave, displayed at 100 mm/sec on a Honeywell Visicorder, were used for measurement of left ventricular ejection time, pre-ejection period, isovolumic contraction time, electromechanical lag, mechanical systole, electromechanical systole, and related ratios. Measurement or calculation of these phases of ventricular systole was performed according to methods described by Tavel.[7] The relationship of these parameters to heart rate was derived from linear regression analysis, using an IBM 7094 computer. Comparisons of cardiac and hemodynamic function in normotensive and hypertensive ponies were made by first matching a

selected group of normal ponies to the group of hypertensive animals by age, sex, body weight, and heart rate. Determination of similarities and differences was then made by comparing paired values for the particular parameters.

RESULTS

General Animal Data

Table I presents physical characteristics of the pony population. The group was approximately evenly divided between males and females, and contained four geldings. The range of weights was rather wide (53.6 kg to 161.5 kg); the average weight, however, was similar to weights reported for three-month-old calves.[2] (Comparative data presented in this section has been obtained from Weber et al.[2] and from Kuida et al.[3] for the calf; from O'Rourke and Bishop[8] for the dog; and from the Handbook of Circulation[9] for man.) Although the ponies ranged in age from 8 months to 9 years, the bulk of the population was 2 to 3 years of age (mean age 1.9 years). Heart weights of the ponies ranged from 4.4 to 7.0 gm per kg of body weight. Similar data for man shows heart weights of 3.7 to 7.5 gm per kg body weight; data for the calf shows 5.7–6.2 gm per kg body weight.

Parameters of hemodynamic function in the ponies are shown in Table II. Average heart rate (during cardiac output determination) was 72.8 beats/min. The rather high rate is no doubt reflective of the manipulations necessary for executing the dye dilution procedure. The range of values for cardiac output was broad (5.2–22.3 L/min), and the mean value (11.1 L/min) was somewhat larger than the largest output value reported for the calf.[2] Cardiac index (L/min/sq meter) and cardiac output per kg body weight were also greater than values reported for the calf, or for man. They were, however, very close to figures found in the conscious dog. Similar comparisons were found for stroke volume, stroke index, and stroke volume per kg body weight.

Systemic blood pressure averaged 144/107 mm Hg in the group of ponies, while pulmonary artery pressure averaged 45/20 mm Hg. Seven of the ponies consistently showed mean aortic pressures of 135 mm Hg or more; they were arbitrarily grouped as "hypertensives," and their data were separately analyzed. The pressures obtained for the group of normal animals were higher than normal for man, but not significantly different from calf or dog. Calculated vascular resistance in the pony was less than that of the calf, reflecting the relatively higher cardiac output in the ponies, with similar blood pressures in the two species.[10] Values for vascular resistance were quite similar to those reported for man. Ventricular work was greater in the pony than in calf, but less than that found in man.

Table III presents ventricular systolic time intervals for the group of ponies. Those measurements which were found to be constant as

TABLE I.—*General Animal Data for the Experimental Pony Population*

Parameter	Range of Values	Mean Value
No. of Ponies		31
Age	8 mo.—9 years	1.9 years
Sex	14 M, 13 F, 4 G	
Body Weight (kg)	53.6 —161.5	94.0
Body Surface Area (sq meters)	1.44 — 6.85	2.18
Heart Weight (kg)	0.298—0.890	0.580
Heart Weight/kg Body Weight (gm/kg)	4.4 — 7.0	6.2

TABLE II.—*Hemodynamic Parameters in Normal Domestic Ponies*

Parameter	Range of Values	Mean Value
Heart rate (beats/min)	38.0 —116.0	72.8
Cardiac Output (L/min)	5.2 — 22.3	11.1
Cardiac Index (L/min/sq meter)	3.18 — 7.98	5.10
Cardiac Output/kg Body Weight (ml/min/kg)	66 — 184	118
Stroke Volume (ml/beat)	44.5 —312.5	151.0
Stroke Index (ml/beat/ sq meter)	45.7 —242.3	69.9
Stroke Volume/kg Body Weight (ml/beat/kg)	0.78 — 2.46	1.64
Systemic (Aortic) Blood Pressure (mm Hg)	120/70—158/100	144/107
Systemic Vascular Resistance (PRU)	0.43 — 1.08	0.70
Left Ventricular Work (joules/stroke)	1.49 — 4.94	2.87
Left Ventricular Ejection Rate (ml/sec/M^2)	101.3 —357.4	209.1
Pulmonary Artery Blood Pressure (mm Hg)	30/10—60/35	45/20
Pulmonary Vascular Resistance (PRU)	0.11 — 0.36	0.19
Right Ventricular Work (joules/stroke)	0.31 — 0.95	0.60

TABLE III.—*Ventricular Systolic Time Intervals in Normal Domestic Ponies*

Parameter	Range of Values	Mean Value
Heart rate (beats/min)	41.9 — 93.0	59.3
Pre-Ejection Period (sec)	0.056—0.129	0.078
Left Ventricular Ejection Time (sec)	0.237—0.453	0.366
Ejection Time Index	0.538—0.644	0.600
PEP / LVET Ratio	0.141—0.311	0.217
Isovolumic Contraction Time (sec)	0.017—0.069	0.035
LVET / ICT Ratio	6.9 — 22.9	10.2
Electromechanical Lag (Q-S1; sec)	0.034—0.060	0.043
Mechanical Systole (S1-S2; sec)	0.271—0.483	0.401
Electromechanical Systole (Q-S2; sec)	0.305—0.543	0.445
Electrical Systole (QT; sec)	0.287—0.476	0.401

heart rate changed (PEP, ICT, Q–S1) were also found to be very similar to values described for man.[11] Parameters which varied with heart rate (LVET, S1–S2, Q–S2, QT) showed similarity to data reported for man as long as the range of heart rates remained close to 70–90 beats per minute. At lower rates, however, these intervals lengthened much more rapidly than those of man, for equal decrements in heart rate. The ETI for the pony was 0.593; that of man is 0.373.[12] Thus the regression line describing the relationship between LVET and heart rate in the pony, beginning as it does at an intercept (ETI) value greater than that found for man, must show a steeper slope if similar LVET values are to be found in the two species at higher heart rates. In fact, LVET is predicted to be identical in pony and man at 88 beats per minute; the slope of the line for the pony is then 0.0036 (negative). For an increase in heart rate of ten beats per minute, LVET in the pony may be expected to shorten by 0.036 sec, while man shortens by 0.013 sec.[13]

An observation of particular interest was the occurrence of apparent spontaneous hypertension (consistent mean aortic pressures in excess of 135 mm Hg) in seven of the ponies studied. Comparison of data available from these animals with data from a selected matched group of normal ponies drawn from the total population showed a number of interesting results.

Table IV presents similarities and differences in hemodynamic parameters in normotensive and hypertensive ponies. The normal group was chosen, in addition to age, sex, and body weight, for similarity in heart rate, in order to minimize effects of rate-dependent variables. Therefore no significant difference in heart rate is found between the two groups. Blood pressures are different, however, with systolic, diastolic, and mean pressures all equally elevated. In all absolute and normalized parameters of cardiac output and stroke volume, the hypertensive group showed increased values. Systemic vascular resistance was approximately equal in the two groups, but left ventricular ejection rate was increased in the hypertensive ponies, as was left ventricular work. Plasma volume showed an apparent increase in the hypertensive group; there were, however, only three determinations in the normal group and only one hypertensive animal was successfully used for this measurement. This combination of hemodynamic changes, that is, elevated cardiac output with no change in vascular resistance, increased left ventricular ejection rate, and equally elevated systolic and diastolic blood pressures has been described in human labile hypertension.[14] The ponies did not, however, show labile pressure changes, but were apparently consistent, at least when measurements were obtained.

Changes were also observed in the hypertensive ponies with regard to ventricular systolic

TABLE IV.—*Comparison of Hemodynamic Parameters in Normal Domestic Ponies and in Ponies with Spontaneously Occurring Hypertension*

Parameter	Normal	Hypertensive	Change %
Heart rate (beats/min)	66.0	65.8	— 0.3
Cardiac Output (L/min)	10.7	13.4	+20.1
Cardiac Index (L/min/sq meter)	4.95	6.35	+28.1
Cardiac Output/kg Body Weight (ml/min/kg)	106	134	+26.4
Stroke Volume (ml/beat)	163	204	+25.1
Stroke Index (ml/beat/sq meter)	75.0	96.5	+28.6
Stroke Volume/kg Body Weight (ml/beat/kg)	1.62	2.05	+27.3
Mean Blood Pressure (mm Hg)	118.6	146.0	+23.1
Systolic Blood Pressure (mm Hg)	146.7	180.7	+23.2
Diastolic Blood Pressure (mm Hg)	99.4	122.7	+23.4
Systemic Vascular Resistance (PRU)	0.68	0.65	— 4.4
Left Ventricular Ejection Rate (ml/sec/M^2)	214	271	+26.6
Left Ventricular Work (joules/stroke)	2.97	4.64	+56.2
Plasma Volume (ml/kg)	50.7	61.7	+21.7

TABLE V.—*Comparison of Ventricular Systolic Time Intervals in Normal and Spontaneously Hypertensive Domestic Ponies*

Parameter	Normal	Hypertensive	Change %
Heart rate (Beats/min)	51.1	49.9	− 2.3
Pre-Ejection Period (sec)	0.076	0.091	+19.7
Left Ventricular Ejection Time (sec)	0.393	0.379	− 3.5
Ejection Time Index	0.576	0.585	+ 1.6
PEP / LVET Ratio	0.193	0.227	+17.6
Isovolumic Contraction Time (sec)	0.037	0.052	+40.5
LVET / ICT Ratio	10.62	7.29	−31.4
Electromechanical Lag (Q-S1; sec)	0.045	0.046	+ 2.2
Mechanical Systole (S1-S2; sec)	0.430	0.431	+ 0.2
Electromechanical Systole (Q-S2; sec)	0.470	0.477	+ 1.5
Electrical Systole (QT; sec)	0.430	0.432	+ 0.5

time intervals. Table V presents this comparative data. Of the intervals measured, only PEP and ICT were altered; both were prolonged. Because Q–S1 remained unchanged, the prolongation of PEP was clearly the result of the increased ICT. This is in contrast to the changes reported in human essential hypertension, where an increased PEP was not due to a change in ICT, but rather to an extended Q–S1.[15]

Table VI contains data describing changes in hemodynamic function and ventricular systolic time intervals in a pony which was found at necropsy to have coarctation of the aorta with bilateral ventricular hypertrophy.[16] This animal is presented here not so much to provide information describing cardiovascular effects of aortic coarctation as to indicate the nature of the animal as a resource in cardiovascular research. The animal showed increased cardiac output, decreased vascular resistance, and reduced ventricular work, prolonged LVET, and a PEP which was shortened by the presence of a decreased ICT.

TABLE VI.—*Changes in Hemodynamics and Phases of Ventricular Systole in a Domestic Pony with Spontaneously Occurring Cardiovascular Disease*

Parameter	Normal Ponies	Diseased Pony
Cardiac Output/kg Body Weight (ml/min/kg)	107	119
Systemic Blood Pressure (mm Hg)	155/105	115/60
Systemic Vascular Resistance (PRU)	0.73	0.27
Left Ventricular Work/kg Heart (joules/stroke/kg)	5.40	2.35
Pulmonary Artery Pressure (mm Hg)	45/20	20/5
Pulmonary Vascular Resistance (PRU)	0.19	0.06
Right Ventricular Work/kg Heart (joules/stroke/kg)	1.20	0.36
Heart Rate (Beats/min)	49.0–50.0	49.8
Pre-Ejection Period (sec)	0.075	0.059
Left Ventricular Ejection Time (sec)	0.396	0.455
Ejection Time Index	0.593	0.639
Isovolumic Contraction Time (sec)	0.036	0.022
Electromechanical Lag (Q-S1; sec)	0.040	0.037
Mechanical Systole (S1-S2; sec)	0.432	0.477
Electromechanical Systole (Q-S2; sec)	0.472	0.514

DISCUSSION

The purpose of this study was the documentation of cardiovascular function in normal, unanesthetized domestic ponies, as a necessary preliminary step in the utilization of the animal as a man-sized animal model for cardiovascular research. Results indicate that for most parameters of cardiovascular function, including both hemodynamic determinations and measurements of ventricular systolic time intervals, the differences among pony, man, and calf are rather minimal.

The dominant difference appears to be the relatively greater cardiac output of the pony. This is not a phenomenon of size difference, as normalized values also demonstrated the increase, and in fact, even though some of the ponies were large (120–150 kg), the mean weight of the group was 94.0 kg, not significantly different from weights reported for three-month-old calves.[2] It is possible that the larger output found in the ponies is a reflection of the general maturity of the group (mean age 1.9 years) as compared with the group of calves. In this regard, it is also interesting to note that heart weights in the two species did not significantly differ in the studies compared (this report; citation 2), even though the ponies were mature and the calves were not.

The fact that differences were so small, in spite of the maturity of the pony group, suggests that the domestic pony may provide an animal model which does not introduce the variable of growth into the conduct of chronic studies. As indicated earlier, the progression of growth can be a problem of major proportions in research involving long term evaluation of a prosthetic device.

Perhaps the most significant observation to be made in this study was that of the occurrence of spontaneous hypertension in a small number of the ponies studied. Such spontaneous hypertension in animals has been observed by Smirk and Hall;[17] more recently Okamoto and Aoki have succeeded in developing an inbred strain of laboratory rats in which spontaneous hypertension is virtually universal.[18] The implications of routine availability of such a preparation for hypertension research are certainly of major importance. The possibility that the domestic pony population contains a large animal model for hypertension research deserves considerable attention and investigation, particularly because the hemodynamic performance of this small group was found to show such marked similarity to hemodynamic function in human labile hypertension.

The relatively young age of these animals (2.2 years for the hypertensive group) suggests that perhaps this hypertension may bear some relationship to juvenile forms of human hypertensive disease. The occurrence of elevated cardiac output in juvenile patients who exhibit labile or intermittent hypertension has been noted.[19] Among the general population of persons with labile hypertensive disease, a high output state with an absence of change in peripheral resistance has been observed.[20-22] Most recently, Frohlich et al.,[14] in studies of labile hypertension, found increased heart rate, systolic and diastolic pressures, cardiac indices, and rate of ejection of the left ventricle. These changes bear a strong resemblance to those observed in the group of hypertensive ponies (see Table IV). Further evidence for early-stage involvement with the disease is the fact that no ventricular hypertrophy was observed in the hypertensive group. This data describes only a small group of animals, and as such does not provide conclusive determination of the nature of hypertension in the domestic pony, nor does it provide an indication of the magnitude of occurrence of the disease in the pony population. It does, however, suggest that further investigation is warranted, in order to exploit what may be a unique opportunity for the expansion of hypertension research.

SUMMARY

The size, maturity, behavior, trainability, and exercise capacity of the pony suggest that it can be an excellent laboratory animal for a broad range of physiological studies. Its resemblance to man in cardiovascular performance indicates in particular that it may be well suited for research in the mechanical assistance of the circulation. The presence of a hypertension syndrome in a small group of ponies suggests that a new resource for hypertension research may become available, if sufficient effort is applied to systematic screening and evaluation of the pony population. Certainly the domestic pony can be a unique resource in cardiovascular research.

ACKNOWLEDGMENTS

Halothane (Fluothane) was generously supplied by Dr. Robert Nuttall, Ayerst Laboratories. The dye dilution cardiac output determinations were made possible by loan of a Waters densitometer; for this assistance the authors are grateful to the Department of Anesthesiology, Baylor College of Medicine. The senior author is particularly appreciative of time allowed from other duties by Dr. Paul A. Klavano, Chairman, Department of Physiology and Pharmacology, College of Veterinary Medicine, Washington State University.

REFERENCES

1. McLaughlin, R. F., Tyler, W. S., and Canada, R. O. A Study of the Sub-Gross Pulmonary Anatomy of Various Mammals Am. J. Anat. 108:149, 1961.
2. Weber, K. T., Dennison, B. H., Fuqua, J. M., Speaker, D. E., and Hastings, F. W. Hemodynamic Measurements in Unanesthetized Calves J. Surg. Res. 11:383, 1971.
3. Kuida, J. T., Brown, A. M., Lange, R. L., and Hecht, H. H. Cardiovascular Studies on Normal Calves Am. J. Physiol. 200:247, 1961.
4. Garner, H. E., Rosborough, J. P., and Amend, J. F. Laboratory Housing for the Pony in an Urban Research Facility Lab. Animal Sci. 21:269, 1971.
5. Rosborough, J. P., Garner, H. E., and Amend, J. F. A Laboratory Restraining Device For the Pony Am. J. Vet. Res. 32:1097, 1971.
6. Liu, C. K. Formulas used in Cardiac Catheterization In Luisada, A., (ed.) *Cardiology*, New York, McGraw-Hill, 1959, pp. 339–346.

7. TAVEL, M. E. *Clinical Phonocardiography and External Pulse Recording*, Chicago, Year Book Medical Publishers, 1967.
8. O'ROURKE, R. A., and BISHOP, V. S. Cardiovascular Hemodynamics in the Conscious Dog Am. Heart. J. 81:55, 1971.
9. DITTMER, D. S., and GREBE, R. M. (eds.) *Handbook of Circulation*, Philadelphia, Saunders, 1959.
10. STOWE, C. M., and GOOD, A. L. Estimation of Cardiac Output in Calves by the Dye and Fick Oxygen Techniques Am. J. Physiol., 198:987, 1960.
11. WEISSLER, A. M., HARRIS, W. S., and SCHOENFELD, C. D. Systolic Time Intervals in Heart Failure in Man Circulation 37:149, 1968.
12. LEIGHTON, R. F., WEISSLER, A. M., WEINSTEIN, P. B., and WOOLEY, C. F. Right and Left Ventricular Systolic Time Intervals: Effects of Heart Rate, Respiration, and Atrial Pacing Am. J. Cardiol. 27:66, 1971.
13. AMEND, J. F., GARNER, H. E., ROSBOROUGH, J. P., and HOFF, H. E. Direct Measurement of Left Ventricular Ejection Time and Ejection Time Index in the Domestic Pony Am. J. Vet. Res. 33: 1569, 1972.
14. FROHLICH, E. D., KOZUL, V. J., TARAZI, R. C., and DUSTAN, H. P. Physiological Comparison of Labile and Essential Hypertension Circ. Res. 27 (Supp. I) :55, 1970.
15. WEISSLER, A. M., HARRIS, W. S., and SCHOENFELD, C. D. Observations on the Delayed First Heart Sound in Mitral Stenosis and Hypertension Circulation 18:165, 1958.
16. AMEND, J. F., GARNER, H. E., ROSBOROUGH, J. P., LEACH, C. S., and HOFF, H. E. Spontaneous Cardiovascular Disease in a Domestic Pony: A Unique Resource in Cardiovascular Research J. Cardiovasc. Surg. 13:181, 1972.
17. SMIRK, F. H., and HALL, W. H. Inherited Hypertension in Rats Nature 182:727, 1958.
18. OKAMOTO, K., and AOKI, K. Development of a Strain of Spontaneously Hypertensive Rats Jap. Circ. J. 27:282, 1963.
19. WIDIMSKY, J., FEJFAROVA, M. H., and FEJFAR, Z. Changes of Cardiac Output in Hypertensive Disease Cardiologia (Basel) 31:381, 1957.
20. EICH, R. H., PETERS, R. J., CUDDY, R. P., SMULYAN, H. I., and LYONS, R. H. Hemodynamics in Labile Hypertension Am. Heart. J. 63:188, 1962.
21. EICH, R., CUDDY, R. P., SMULYAN, H., and LYONS, R. H. Hemodynamics in Labile Hypertension: A Follow-up Study Circulation 34:299, 1966.
22. JULIUS, S., and CONWAY, J. Hemodynamic Studies in Patients with Borderline Blood Pressure Elevation Circulation 38:282, 1968.

DISCUSSION

CHAIRMAN HAWTHORNE: That's a very excellent paper, Dr. Amend. I'd like to ask you a question. If, as you showed, the stroke volume and mean pressure were increased and the heart rate unchanged in your hypertensive ponies, why wasn't the peripheral resistance changed?

DR. AMEND: Because as the flow increased the pressure increased proportionally.

DR. HAWTHORNE: I don't see how you could calculate a peripheral resistance which was not increased. But it's still an interesting point.

JOHN T. REEVES, University of Kentucky, Lexington, Ky.: I think in most people with hypertension, the cardiac output is not increased. I can't cite a reference, but there is a group with increased output that are known to have "hyperkinetic circulation." They tend to have tachycardia in addition to the high output, and, in general, the syndrome seems to be reversed by beta blocking drugs. Have you used beta blocking drugs in the equine species and what, if any, effects?

DR. AMEND: There's only been one report of the application of beta blocking drugs in equine species. It was the report of Tavernor and Lees in the horse. Their report was simply a general evaluation of the effects of propranolol in the horse. I wanted to try beta blocking agents in these hypertensive ponies and never had an opportunity so I really can't speak more about it. We did match our groups in terms of heart rate specifically for the purpose of matching systolic time intervals. Therefore, we lost the opportunity to see whether or not the ponies in fact had increased heart rate. As I recall, over the total normal group, they were not particularly changed, even though heart rates were not matched.

COMPARATIVE MECHANICAL BLOOD PROPERTIES

P. L. Blackshear and R. J. Forstrom*

The red cells in mammalian and non-mammalian species display a range of size, shape, membrane flexibility, tendency to aggregate and intracellular viscosity characteristic of each species. These parameters influence the ability of the cells to resist mechanical trauma, and along with the properties of the plasma, influence the rheological behavior of the blood. In this paper, (1) the relevant mechanical properties of the erythrocytes of several species obtained in the laboratory of Shu Chien are summarized along with the resulting rheological influences. (2) The mechanical hemolysis results of Forstrom's jet test for several species are summarized in the light of the mechanical properties of the erythrocytes. (3) The role of cell size and deformability in influencing the diffusion of extracellular solutes in the presence of a velocity gradient is calculated (but not demonstrated experimentally). (4) The role of cell size and deformability in influencing the cell concentration distribution near walls during flow is qualitatively predicted (but not demonstrated experimentally). (5) The way these factors determine the degree to which model experiments can be related to human blood is summarized.

INTRODUCTION

Shu Chien, et al[1] have combined the microrheological considerations pioneered by Goldsmith and Mason,[2] Cokelet,[3] Rand,[4] and others with the cell deformability determinations of Weed, et al[5] and LaCelle and Weed[6] and with supplemental experiments have formulated a set of defined properties of cells that aid greatly in understanding the macrorheological behavior of blood of various mammalian and non-mammalian species.

Because this work is so germaine to the present topic we will, with Professor Chien's permission, review the useful microrheological and physical properties of several species published by him. We find that Chien's defined quantity, the sphericity ratio, helps greatly in classifying the strong dependence of cell lysis stress on cell volume reported in the Forstrom jet test.[7]

* Bioengineering Laboratory, Department of Mechanical Engineering, University of Minnesota, Minneapolis, Minnesota.

Diffusion induced by cell motion predicted by the model experiments of Collingham[8] and Singh[9] and measured in blood by Overcash and Keller,[10] also is dependent upon cell size and sphericity ratio. The cell depleted layer near a wall in flowing blood should be similarly dependent upon these two cell properties.[11–13]

These mechanical blood properties will be discussed in the sections that follow. We will show that size, shape and deformability on which these properties depend are themselves apparently interrelated. Thus, *red blood cell volume alone* may well be the single quantity required to predict the mechanical blood properties considered.

COMPARATIVE MACRORHEOLOGICAL BEHAVIOR OF BLOOD AND ITS DEPENDENCE UPON RED BLOOD CELL PROPERTIES

In this section we will review some of the results of Chien and his collaborators as published in ref. (1). Figures 1–7 in this section are from ref. (1).

The shear rate dependence on viscosity of several mammalian and non-mammalian species is shown in Figure 1. At low shear rates, the viscosity of mammalian blood falls into one of two groups, whereas at high rates of shear the results converge. (Note: hematocrit is not the same in this comparison.)

Cell suspensions of equal hematocrit display viscosities that depend upon shear rate and apparently upon the volume swept by the cell (or rouleaux) as it rotates.[1] This volume is thought to be a function of cell or rouleaux size, shape, and resistance to deformation. These influences on the viscosity of human blood are shown in Figure 2. Starting from a low shear rate of 10^{-2} sec^{-1}, large rigid rouleaux make human blood nearly 10 times as viscous as when

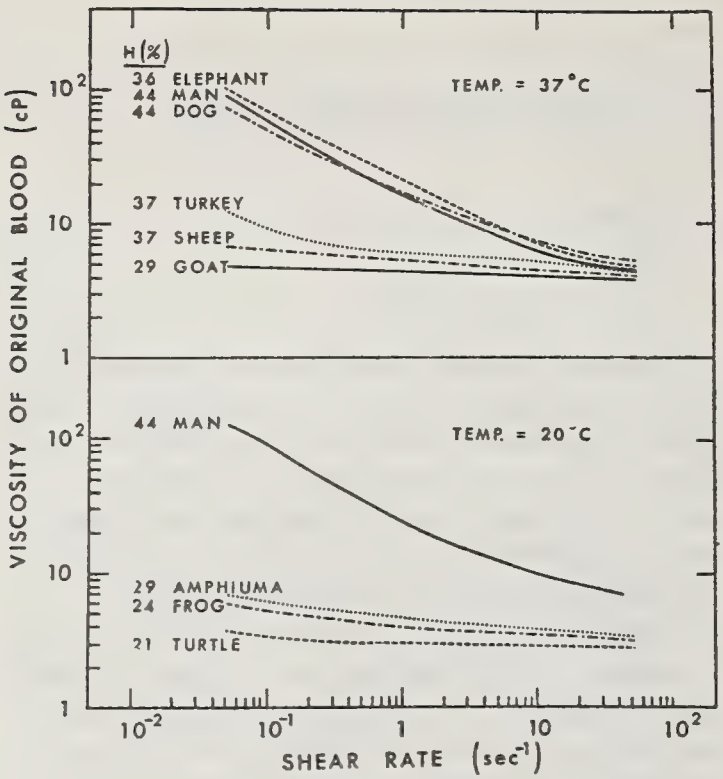


FIGURE 1.—Relation between viscosity of original whole blood and shear rate. (from ref. 1).

cells are dispersed. As shear rate increases, fluid forces cause rouleaux to flex (so they have a smaller effective size) and disperse. At low shear rates individual cells appear rigid. As shear rate increases further, rouleaux are further dismantled and individual cells begin to flex—sweeping a smaller effective volume as they rotate.

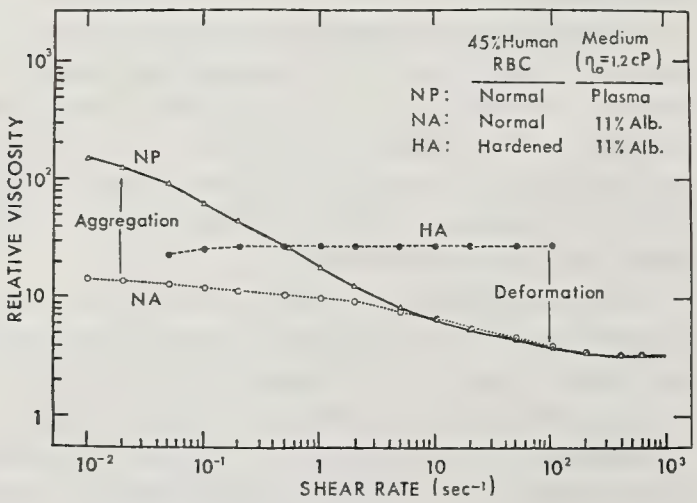


FIGURE 2.—Relation between relative viscosity and shear rate of human normal RBC in plasma, normal RBC in 11% Albumin-Ringer, and hardened RBC in 11% Albumin-Ringer. (from ref. 1).



FIGURE 3.—Election micrograph of RBC suspended in Dextran with a mean molecular weight of over one million. (from ref. 1).

Interestingly, the ability to form rouleaux depends upon cell deformability. Figure 3 shows how cells must deform in order to form rouleaux. The ability to deform is thought to be related to the surface to volume ratio, membrane flexibility and perhaps to internal viscosity.

An idea of size and shapes of the cells tested can be obtained from Figure 4 (note goat cells do not spontaneously form rouleaux). Average statistics of cells examined are given in Table I. Note the mean corpuscular hemoglobin concentration (MCHC) varies but little suggesting that in isotonic suspensions the internal viscosity will not vary from species to species. Also note the sphericity index appears to be a monotonic function of cell volume. More will be said of this in the next section.

Cell deformability is classified by filter tests in Figure 5. Note the results for the mammalian cells are grouped closely together despite large cell diameter variations. This is because the small cells are less deformable due to, for the most part, their high sphericity index. Similarly high sphericity index impairs the tendency to form rouleaux. This can be seen in Figure 6. The

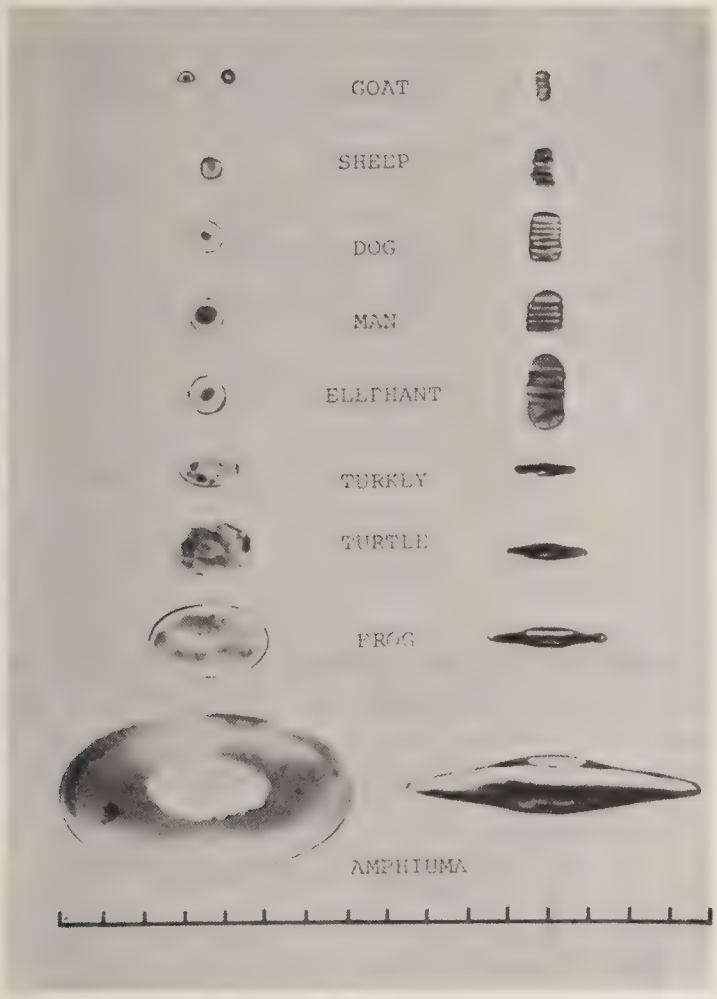


FIGURE 4.—Photomicrographs of erythrocytes from nine animal species at the same magnification (10 μ scale at bottom). Edge view of mammalian RBC is that for rouleaux in autologous plasma except goat RBC, for which rouleaux were formed in 2% Dextran (M. W. $\sim 10^6$) solution. In non-mammalian RBC, nuclei are visible in both views. (from ref. 1).

shear dependence parameter is defined as the difference in viscosities at a high (5.2 sec^{-1}) and a low (0.52 sec^{-1}) shear rate divided by the viscosity at the high shear rate. The relationship between shear dependence and tendency to aggregate is shown in Figure 7 versus fibrinogen level.

Recall from Figure 2 that the two factors which determine the shear dependence of viscosity are cell deformability and tendency to aggregate. But from the above we see in mammalian RBC the tendency to aggregate is also a function of cell deformability. Finally recall that deformability is related to sphericity index which in turn appears to be a monotonic function of cell volume. Thus the rheological behavior of the blood of an experimental mammal should be predictable by a knowledge of plasma viscosity, hematocrit and cell volume.

COMPARATIVE STRENGTH OF MAMMALIAN RED BLOOD CELLS

In this section we will attempt to show that the membrane stress at which hemolysis occurs

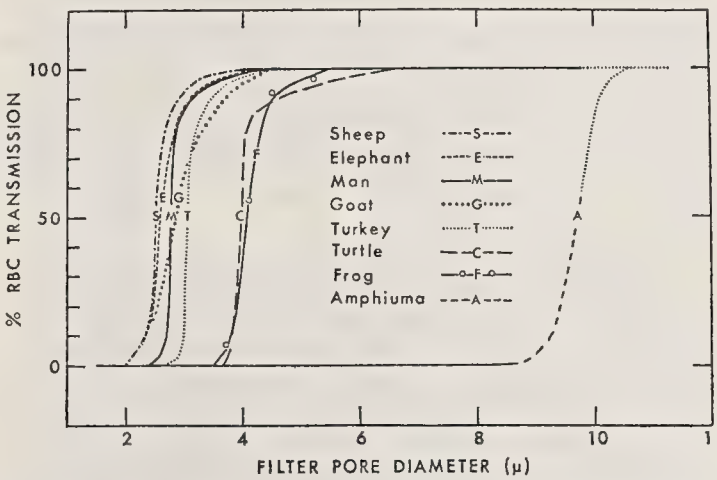


FIGURE 5.—Relation between normal RBC transmission through polycarbonate sieves and sieve mean pore diameter (from Ref. 1).

TABLE I.—*Dimensions of Erythrocytes (from Shu Chien, et al.)*

	Diam. × Diam. × Thickness ($\mu \times \mu \times \mu$)	Surface area (μ^2)	Volume (μ^3)	Sphericity Index ($4.84\ V^{2/3}/A$)	Nucleus ($\mu \times \mu$)	MCHC (g%)
Mammals						
Goat	3.0–4.5 × 1.6	42	18	0.80	—	35.6
Sheep	4.8 × 4.8 × 1.7	60	37	0.78	—	35.2
Dog	7.5 × 7.5 × 1.6	126	74	0.65	—	34.8
Man	8.4 × 8.4 × 1.6	152	90	0.64	—	33.5
Elephant	9.2 × 9.2 × 1.7	181	112	0.62	—	36.2
Non-mammals						
Turkey	13.9 × 8.0 × 2.5	249	136	0.51	6.0 × 3.0	33.6
Turtle	19.7 × 12.7 × 3.5	513	394	0.51	6.6 × 4.3	33.5
Frog	28.0 × 15.6 × 6.0	1102	823	0.38	10 × 6.7	28.5
Amphiuma	65.0 × 30.0 × 13.5	6605	10,800	0.36	24 × 15	26.2

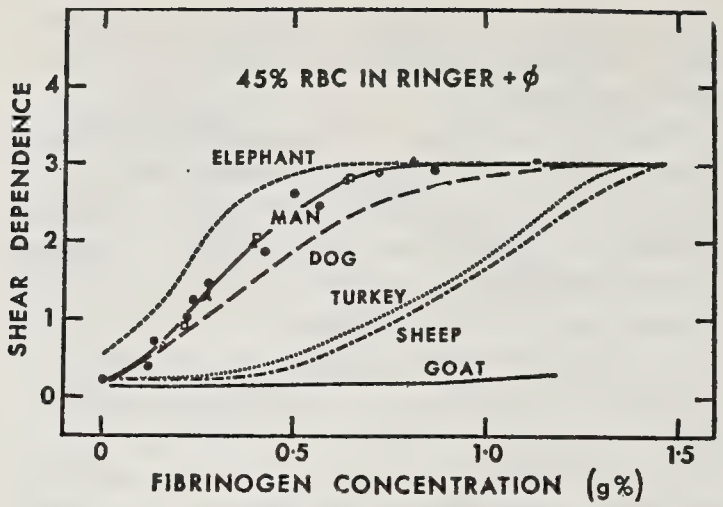


FIGURE 6.—Influence of fibrinogen concentration on shear dependence of viscosity of 45% cell suspension in Ringer solution + fibrinogen. The curve for man was obtained by adding purified fibrinogen from elephant (Δ), man (●) and goat (□) to human RBC. In all other species autologous, purified fibrinogen was used (from Ref. 1).

as a result of a suddenly applied force is a constant for all mammalian RBC. Forstrom[7] has designed and tested a device called the jet fragility test to explore the strength of RBC. Saline is rapidly jetted through a 23 gauge needle into an initially stagnant reservoir of blood (see Figure 8).

Results of a typical experiment are shown in Figure 9. In this test, 2 cc of saline were jetted into 1 cc of human blood reduced in hematocrit with saline to 8%. We choose to characterize a blood sample by the critical velocity, U_c, the velocity corresponding to the intersection of the line at zero hemolysis. Here, U_c is 2100 cm/sec and the flow is turbulent (Reynolds number of about 7000). The mechanism of jet lysis is thought to be related to either turbulent shear stresses or pressure fluctuations, both having a value about $\tau = 0.017\ \rho U_c^{2}$.[14]

Data for seventeen different mammals are summarized in Table II and plotted in Figure 10 as critical velocity versus cell volume. The data are represented reasonably well by the relation $U_c^2\ V$ = constant, which can be expressed as τV = constant. This rupture stress relation will now be related to the cell membrane tension.

As we have seen above, static cell dimensions

TABLE II.—*Jet Fragility Test Results*

Mammal (#)	(Volume (μ^3)	U_c (cm/sec)
Human Cord (12)	103	1930
Human Female (14)	84	2120
Human Male (26)	84	2240
Hamster (10)	62	2260
Dog (19)	72	2310
Raccoon (1)	44	2310
Rabbit (3)	70	2330
Squirrel Monkey (2)	63	2380
Brown Bear (1)	52	2390
Guinea Pig (1)	76	2460
Rat (10)	64	2470
Pig (1)	58	2700
Mouse (10)	48	2800
Siberian Tiger (1)	53	3070
Domestic Cat (1)	39	3150
Horse (1)	37	3510
Cow (2)	32	3850
Sheep (4)	31	3880
Goat (1)	24	5800

do not characterize a flowing red blood cell. In velocity gradients, RBC are distorted and deform into shapes we may approximate by a cylinder with hemispherical ends but with the

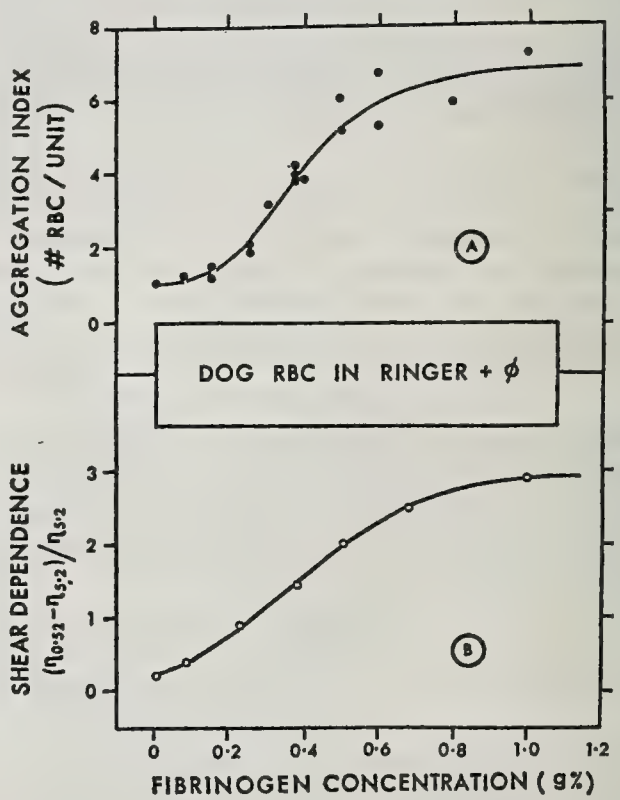


FIGURE 7.—(a) Influence of fibrinogen concentration on RBC aggregation. In 0.5% cell suspensions the average number of RBC in each aggregation unit was calculated. (b) Influence of fibrinogen concentration on shear dependence of viscosity of 45% cell suspensions (from Ref. 1).

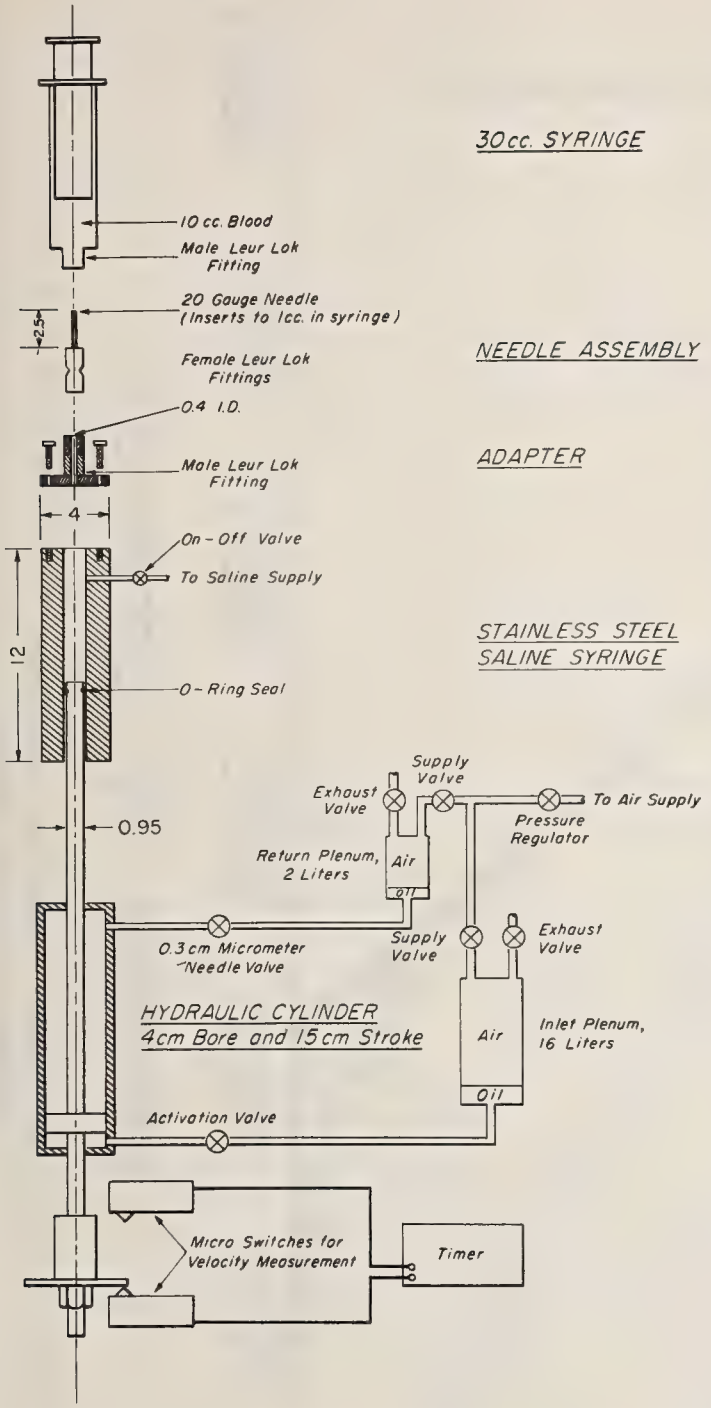


FIGURE 8.—Schematic representation of jet fragility test facility.

volume and area of the undistorted cell. In such a flow, we propose the criteria for lysis is $\tau A = 2\pi R\sigma$, where A is cell area, R is cylinder radius, and σ is the membrane tension in dynes/cm. For all RBC, we expect A and V of the deformed cell to equal the static values.[15] Letting L′ be the length of the cylindrical portion of the cell, $A = 2\pi RL' + 4\pi R^2$ and $V = \pi R^2 L' + 4/3\ \pi R^3$.

We have plotted Chien's data of cell area and volume for different species (Table I) and noted a surprising relationship (see Figure 11). Even for the nucleated non-mammalian RBC, a unique relation, $A = 3.9V^{0.81}$, adequately describes the data. For comparison purposes, the relation $A = 4,84\ V^{2/3}$ describes a sphere. The equation $A = 1.6V$ can describe four of the five mammalian cells. (The sphericity index,[1] proportional to $V^{2/3}/A$, may also be used to describe the data. Letting $A \propto V^n$, the index is proportional to $V^{2/3-n}$. The exponent, n, is greater than 2/3, so the sphericity index increases monotonically as cell volume decreases.) By using the area-volume relation and solving for R and L′, we find R is virtually a constant and has a value of about 1.2μ for all mammalian cells. This finding is not unexpected in reviewing the RBC transmission through sieves in Figure 5.

Our force balance becomes $\sigma = \tau\ A/\ (2\pi R) \propto \tau A \propto \tau V^n$, where exponent n is about 1.0. We recall the jet result that τV = constant and thus σ = constant. To our knowledge, this is the first demonstration that all mammalian species may be endowed with RBC of the identical strength.

The cylindrical model of the deformed cell aids in the clarification of other jet fragility results. RBC were tested in various tonicities and the results are shown in Figure 12. Ordinate τ' is the ratio of hemolytic turbulent stress to the stress at tonicity 1.0. Note that the RBC in an isotonic environment are the most susceptible to fluid stresses.

Assuming (1) the cell area is 152μ^2 and is independent of tonicity, (2) the isotonic volume is 90μ^3, and (3) the osmotic volume change relation is $V = 90\ [(0.6/T) + 0.4]$, then R and total deformed length $L = L' + 2R$ may be calculated. These dimensions are shown in Figure 13 relative to the values at tonicity 1.0.

In cases for which the areas is constant, $\sigma \propto \tau/R$. Figures 12 and 13 are combined in Figure 14 to examine this relation. Lethal membrane tension appears to be constant in osmotic swelling. However, the lethal tension increases with shrinking. The isotonic suspension appears to separate the two states of the RBC. It is un-

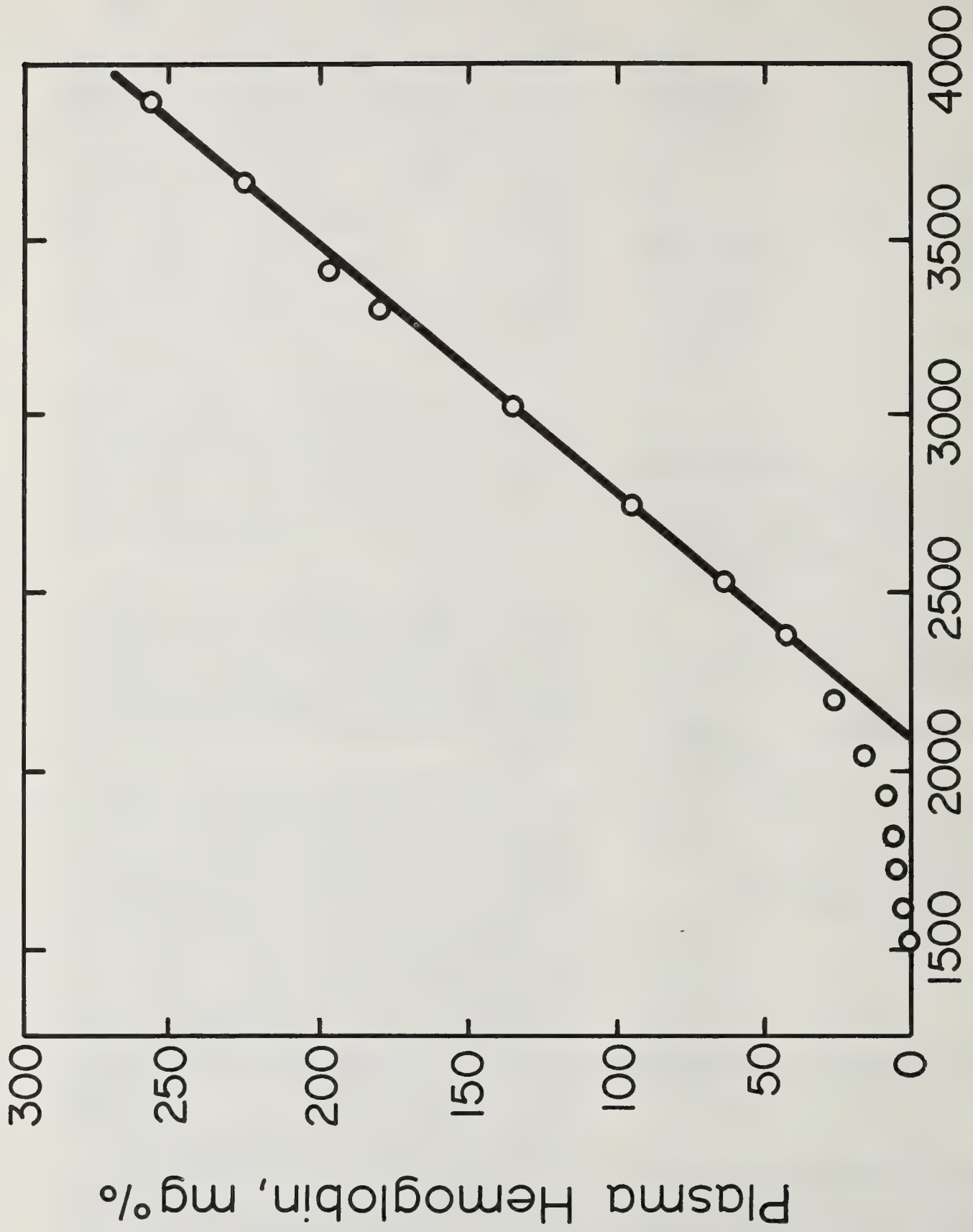


FIGURE 9.—Representative hemolysis data for saline injection into human blood. The interception of the line with the abscissa is the critical velocity, U_c.

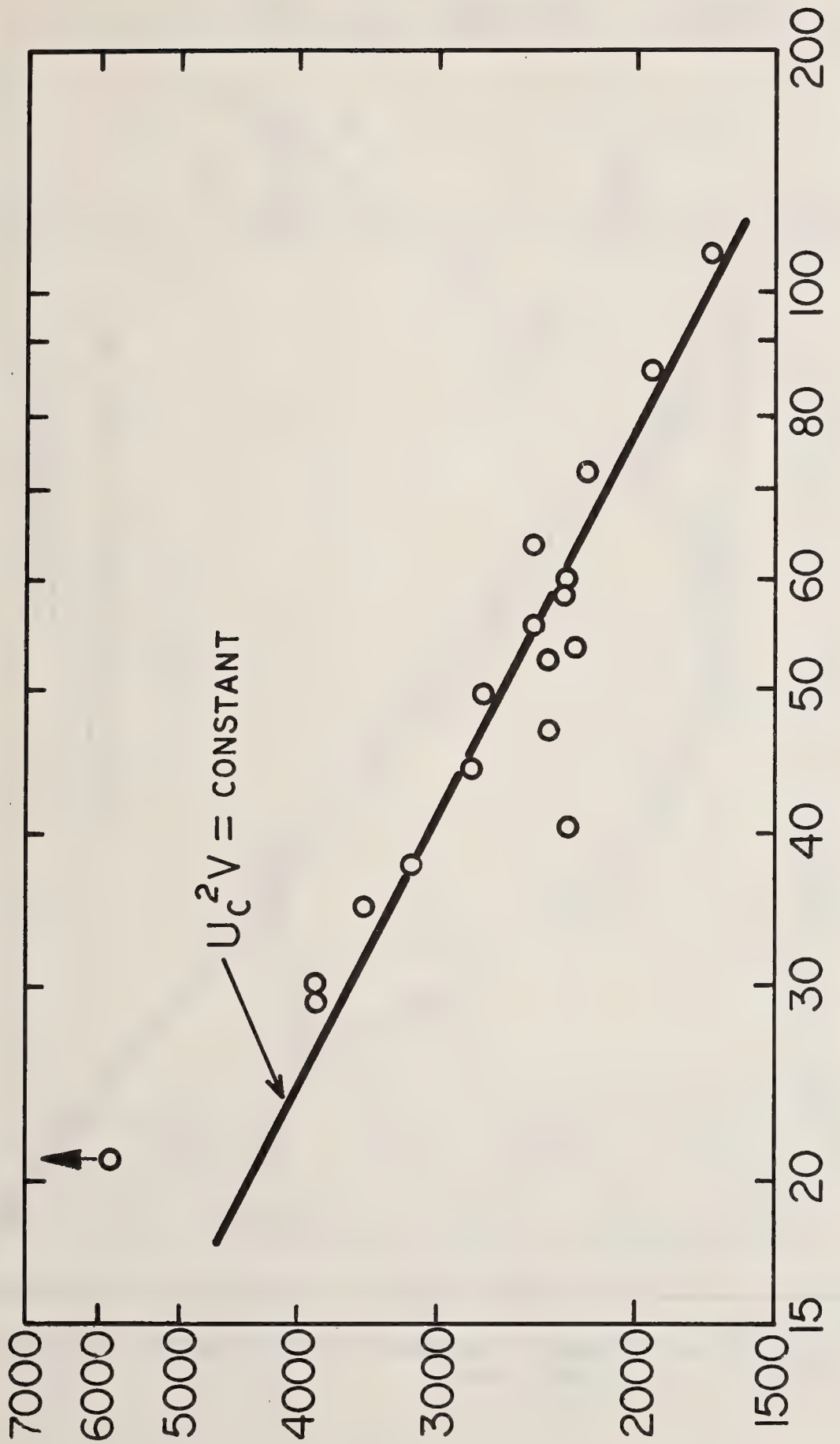


FIGURE 10.—Relation between critical velocity and RBC volume for mammalian RBC listed in Table II. Note the data can be represented by U_c^2V = constant.

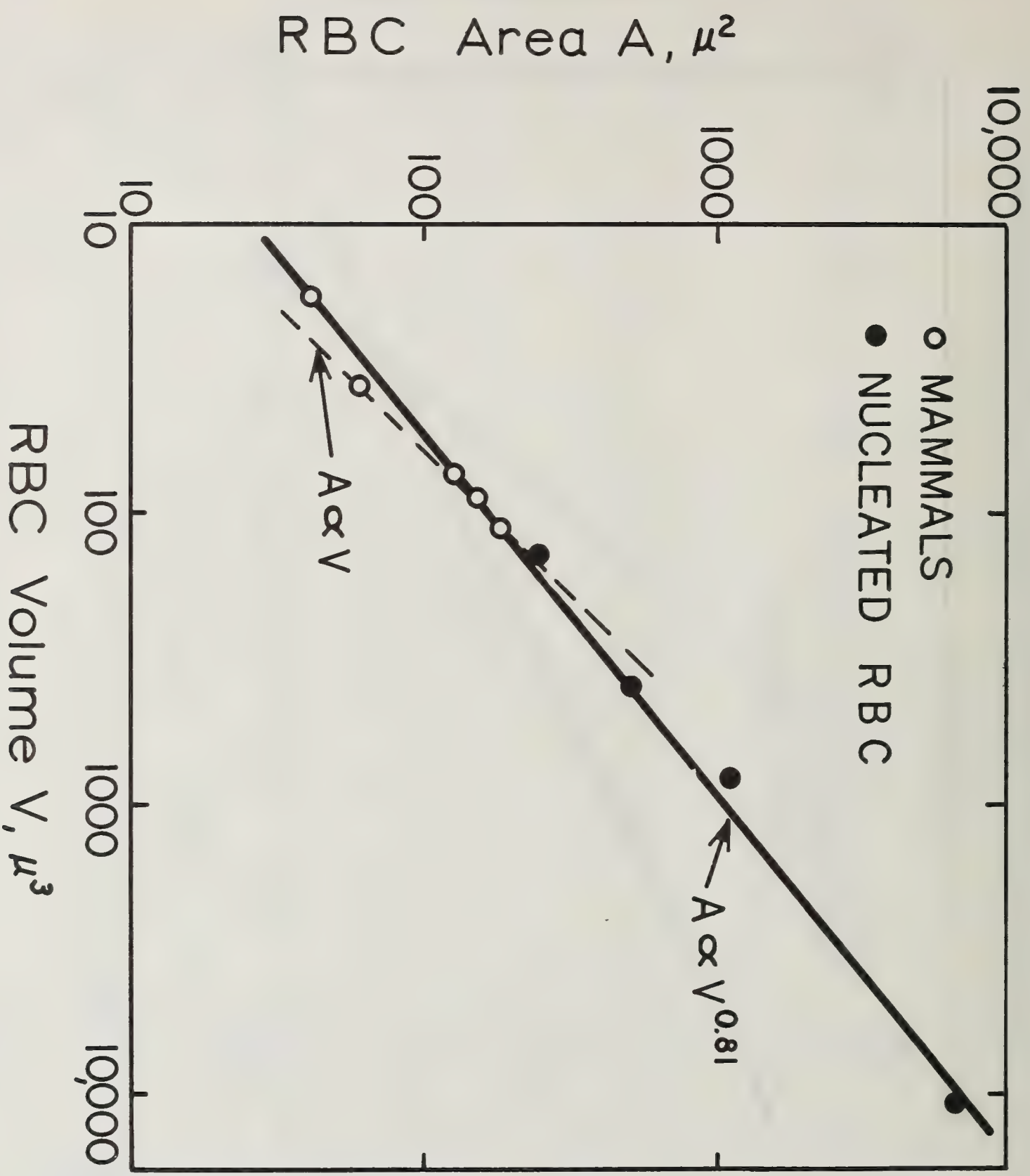


FIGURE 11.—Relation between RBC area and volume of five mammalian and four non-mammalian species. (data is from Table I).

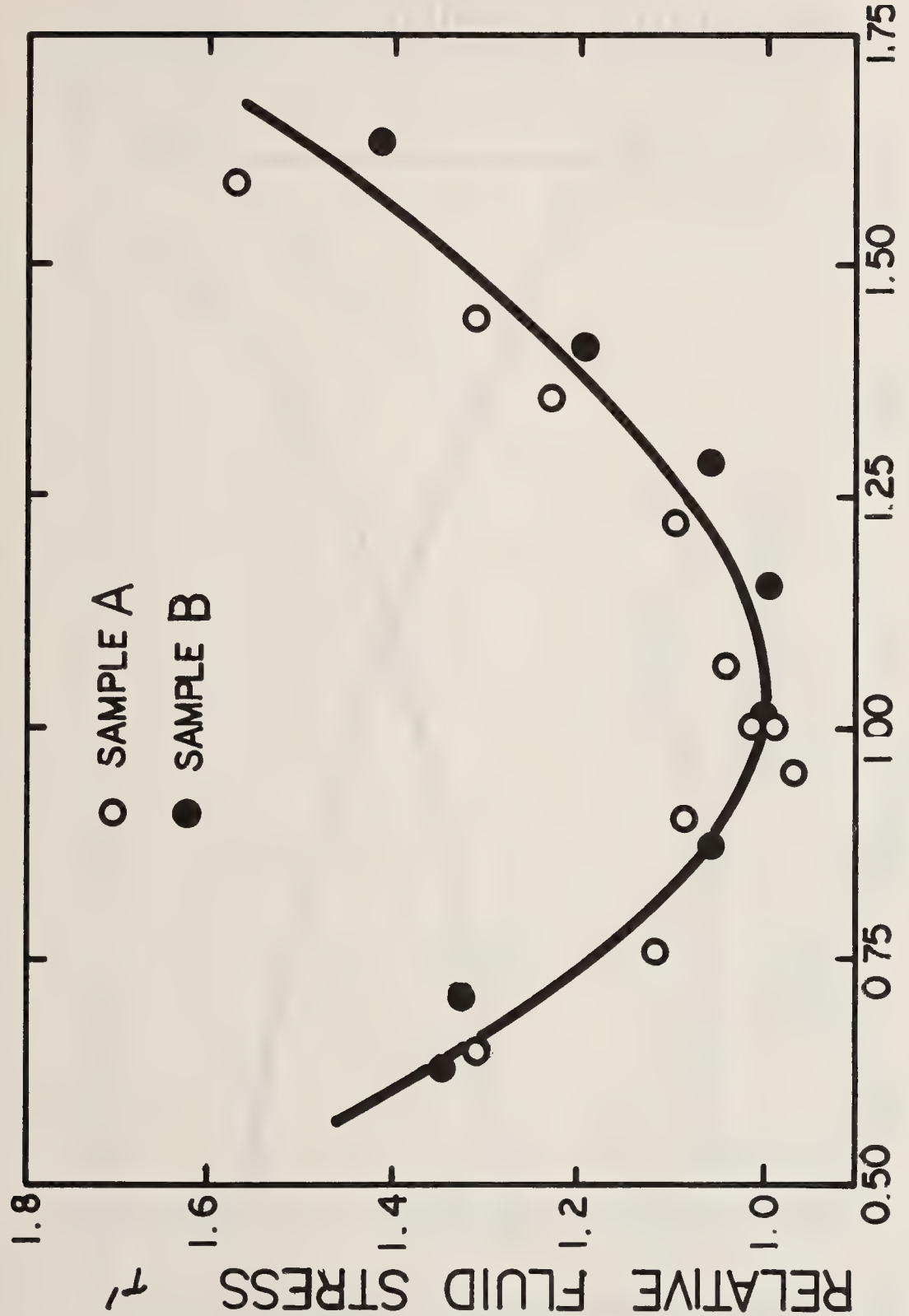


FIGURE 12.—Relation between fluid stress relative to tonicity 1.0 and tonicity in osmotic volume change experiments.

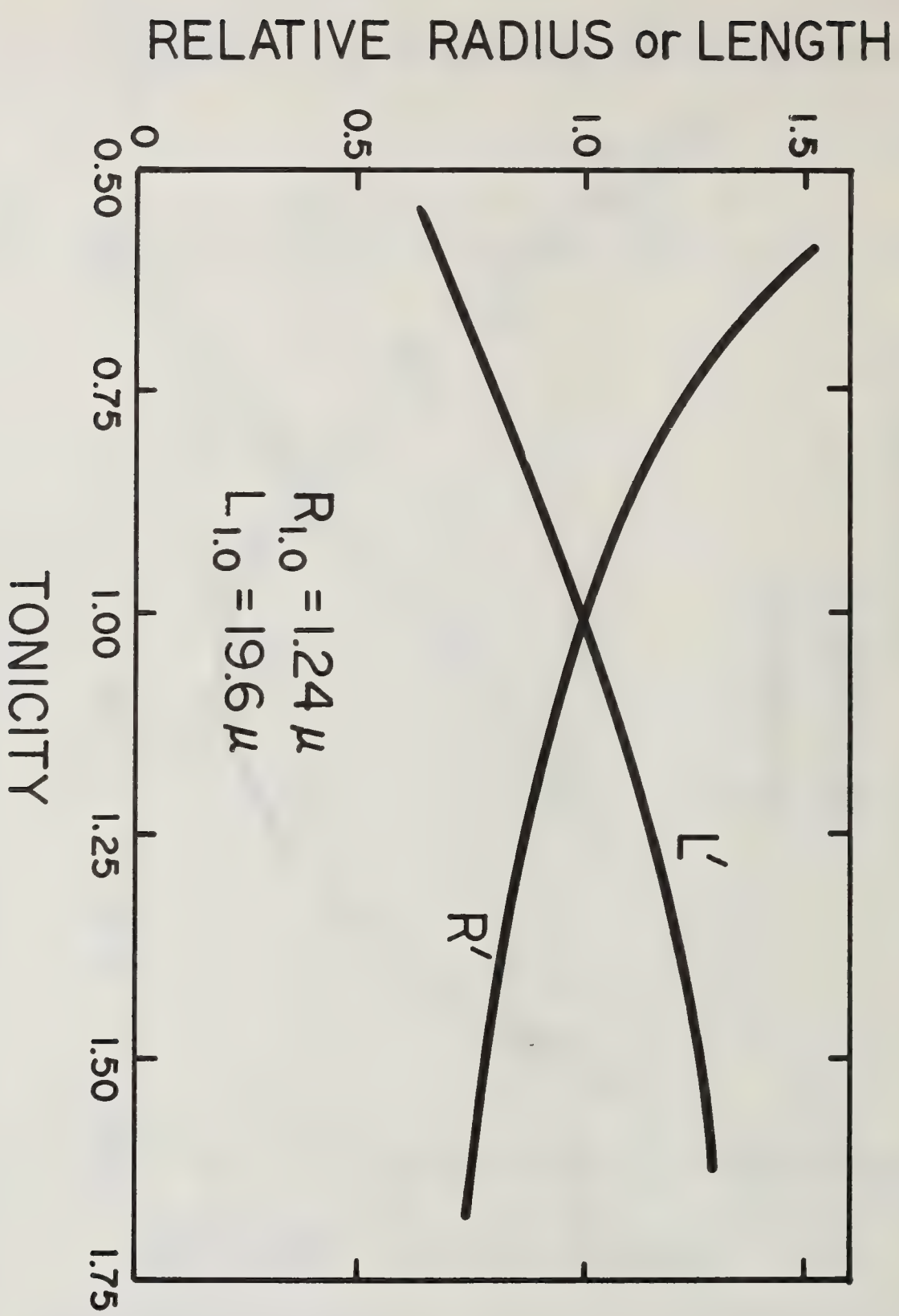


FIGURE 13.—Calculated radius and length relative to dimensions at tonicity 1.0 of a RBC in the shape of a cylinder with hemispherical ends of surface area of 152 μ^2 and isotonic volume of 90 μ^3.

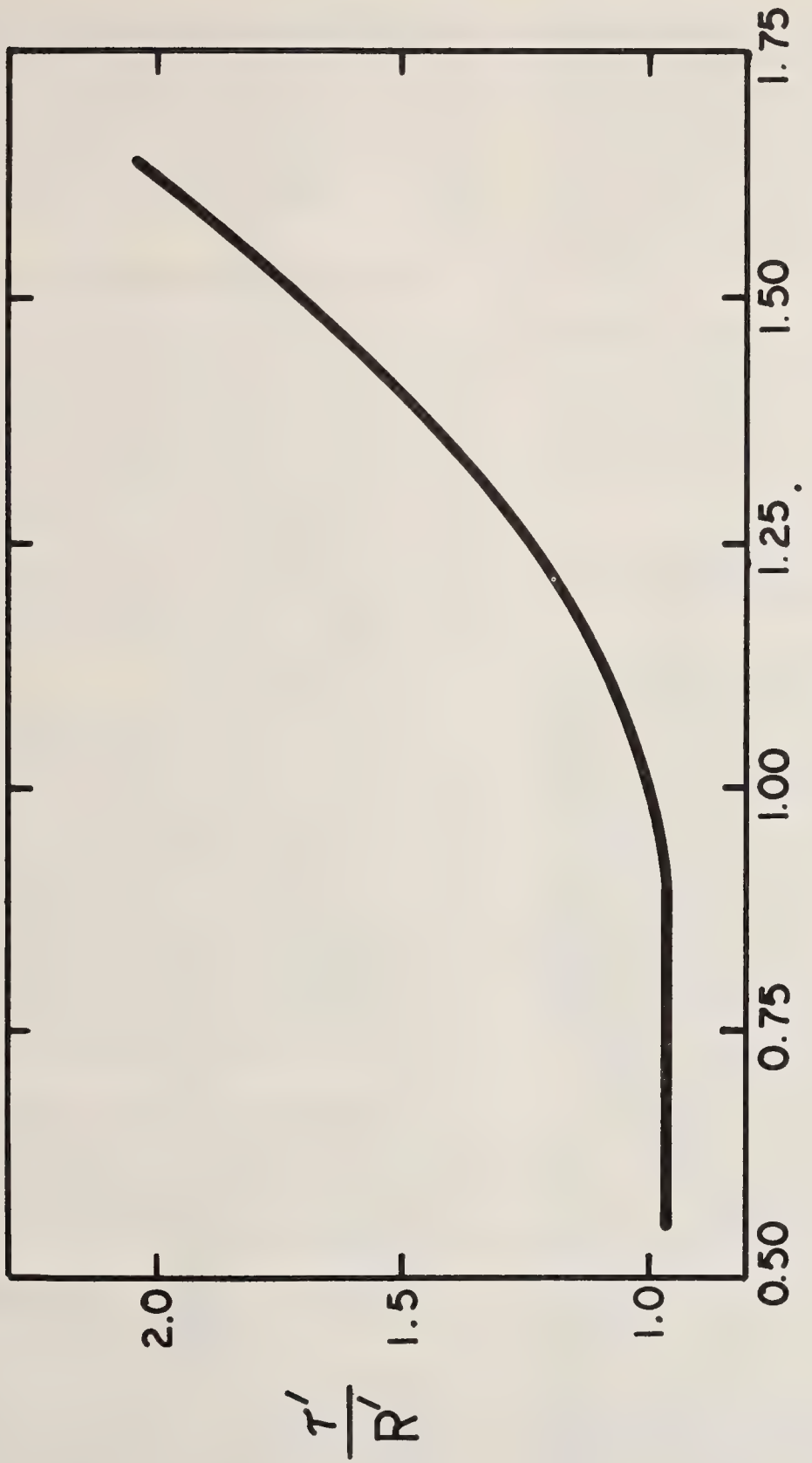


FIGURE 14.—Average data of figure 12 replotted as τ'/R'. A constant value of the ordinate indicates a state of constant membrane tension at lysis.

clear as to why hypertonic suspensions increase the apparent lethal tension. At isotonic conditions the fluid shear stress of short (less than 1 sec) duration that any mammalian red cell can withstand can be estimated from $\tau V =$ constant $\approx 7 \times 10^{-2}$ dyne-microns (10^{-8} dynes/cm^2 $\times$ microns^3). For stress of long duration (>15 sec) the lethal stress should be half the above value.[4]

For cell failure where the lethal membrane tension is required (e.g. trapped cells, filter lysis, etc.) a lethal membrane tension of $\sigma =$ 30 dynes/cm for short duration (t <1 sec) and 15 dynes/cm for long duration (t >15 sec) can be used for *all* mammalian species.

MASS TRANSPORT AUGMENTATION BY RED BLOOD CELL MOTION

Diffusion augmentation of oxygen by red blood cell motion in a velocity gradient has been reported by Keller.[10] Augmentation of platelets, white cells and other plasma solutes also depend on RBC motion.[16,17] Predictions based on model studies for diffusion augmentation have been published by Collingham.[8] Figure 15 from his thesis permits an estimate of the range of importance of this effect. Simply read up from molecular diffusion coefficient (or particle weight of solute) to the local shear rate. If the intersection occurs above the 10% augmentation line an effect should be realized.

In this section we wish to speculate on the influence of species differences on this effect. Collingham's data[8] show that in model studies diffusion augmentation varies roughly as particle diameter. Thus, if the red blood cells were all rigid, species differences in augmentation would be large. In the limit that the cells distort into cylindrical shapes nearly parallel to the wall, the effect would depend on cylinder radius. We have seen in the section above that this radius is roughly the same for all mammals (about 1.2μ). Thus, augmented diffusion would be the same for all species at high shear rates where distortion can be expected.

Until this diffusion phenomenon is studied experimentally (it is much too difficult to treat analytically now) it would seem that model experiments at very high wall shear rates may predict diffusion augmentation for humans that would be the same or in proportion to cell size. In any event, to the extent that diffusion augmentation by red cell motion is understood at present it appears likely that the species differences if they exist will correlate with *cell size.*

CELL CONCENTRATION NEAR WALLS

Bugliarello, et al[11,12] show that the mean distance in microns of the nearest cell to the vessel wall can be represented by 100/Hct for human cells. Goldsmith[18] shows that cells in dilute cell suspensions are repelled from the wall strongly and move to the tube axis; hardened cells are less strongly repelled. Thus, it is inferred that the cell depleted layer near walls is determined by a balance of forces, repulsion due to a wall effect and dispersion due to cell-cell interactions in a velocity gradient.

Brenner predicts the wall repulsion force to be proportional to velocity gradient squared.[13] Voss confirms this experimentally (see ref. 19). Brenner also predicts wall repulsion varies directly with deformability and as diameter to a power greater than 2. Physical arguments suggest that dispersion forces should decrease with increased deformability and be independent of cell diameter. As we have seen deformability is related to cell size so that all the factors that influence skimming layer depend upon size.

All these effects conspire to reduce the cell depleted layer as cell volume decreases. The increased cell wall interaction and enhanced mass transfer that results may be of significance in model experiments.

RELATION OF MODEL EXPERIMENTS TO HUMAN BLOOD

The red blood cell of man is one of the largest mammalian cells. In relation to the research animals—goat, sheep, cow, rabbit, and dog, the human RBC is the largest. The following discussions, thus, will assume the RBC of all model experiments is smaller than the human RBC.

At shear rates exceeding 50 sec^{-1}, the viscosity of the model and human blood is about the same at normal species hematocrit. At low

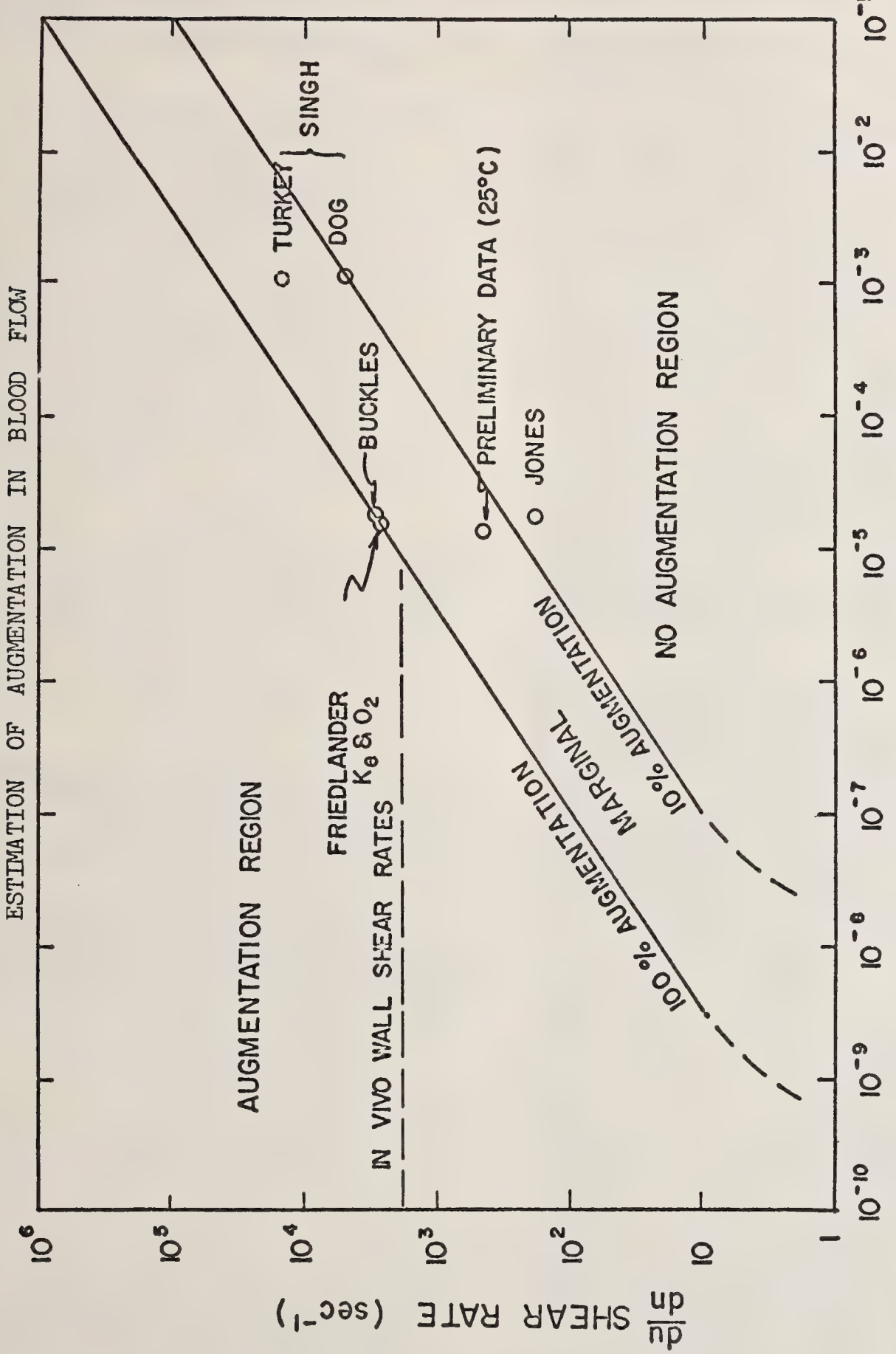


FIGURE 15.—Estimation of augmentation in blood flow.

shear rates (<10 sec^{-1}), blood viscosity depends strongly on shear rate and human blood viscosity may exceed model viscosity by more than a factor of 10. Plasma fibrinogen concentration is important at low shear rates.

Cell deformability is related to the sphericity index because spherical cells, if of fixed surface area, are capable of no deformation. Evidence was presented that the index is a function of cell volume, and the RBC of man is more deformable than model cells. The capability to deform enhances rouleaux formation, increases cell vulnerability, and permits cells to penetrate holes much smaller than their disc diameter.

Augmented diffusion due to rigid red cell rotation and collision is expected to increase linearly with cell size. However, qualitative considerations suggests cell induced diffusion diminishes with increased deformability, the latter increasing with cell size. Model experiments are expected to slightly underestimate augmented diffusion in human blood and the diffusion rate should be proportional to the ratio of cell diameter to a power between one and zero.

Size and deformability conspire in producing the cell depleted layer. Model experiments are expected to underestimate the extent of the layer in human blood.

Recalling the fact that red blood cell properties are interrelated, from the standpoint of the mechanical properties of blood the animal that best models man is one whose *red blood cell volume* is the same as that of man.

SUMMARY

The red cells in mammalian and non-mammalian species display a range of size, shape, membrane flexibility, tendency to aggregate and intracellular viscosity characteristic of each species. These parameters influence the ability of the cells to resist mechanical trauma, and along with the properties of the plasma, influence the rheological behavior of the blood. In this paper:

(1) The relevant mechanical properties of the erythrocytes of several species obtained in the laboratory of Shu Chien are summarized along with the resulting rheological influences.

(2) The mechanical hemolysis results of Forstrom's jet test for several species are summarized in the light of the mechanical properties of the erythrocytes.

(3) The role of cell size and deformability in influencing the diffusion of extracellular solutes in the presence of a velocity gradient is calculated (but not demonstrated experimentally).

(4) The role of cell size and deformability in influencing the cell concentration distribution near walls during flow is qualitatively predicted (but not demonstrated experimentally).

(5) The way these factors determine the degree to which model experiments can be related to human blood is summarized.

REFERENCES

1. Chien, S., Usami, S., Dellenback, R. J., and Bryant, C. A.: Comparative Hemorheology—Hematological Implications of Species Differences in Blood Viscosity, Biorheology, 8:35, 1971.
2. Goldsmith, H. L., and Mason, S. G.: The Microrheology of Dispersions, in Rheology, Vol. 4 (Edited by Eirich, F. R.) p. 850, Academic Press, New York, 1967.
3. Cokelet, G. R. and Meiselman, H. J.: Rheological Comparison of Hemoglobin Solutions and Erythrocyte Suspensions, Science 162: 275, 1968.
4. Rand, R. P.: Mechanical Properties of the Red Cell Membrane. II. Viscoelastic Breakdown of the Membrane, Biophys. J., 4:308, 1964.
5. Weed, R. I., LaCelle, P. L., and Merrill, E. W.: Metabolic Dependence of Red Cell Deformability, J. Clin. Invest., 48:795, 1969.
6. LaCelle, P. L. and Weed, R. I.: Alteration of Membrane Deformability in Hemolytic Anemias, Seminars in Hemat., 7:355, 1970.
7. Forstrom, R. J.: Ph.D. Thesis, Minneapolis, University of Minnesota, 1969.
8. Collingham, R. E.: Ph.D. Thesis, Minneapolis, University of Minnesota, 1968.
9. Singh, A.: Ph.D. Thesis, Minneapolis, University of Minnesota, 1968.
10. Keller, K. H.: Effect of Fluid Shear on Mass Transport in Flowing Blood, Fed. Proc., 30:1591, 1971.
11. Bugliarello, G.: Hemodynamic and Mass Transfer Implications of Phase Separation Phenomena in the Microcirculation, AGARD, Conf. Proc. No. 65 on Fluid Dynamics of Blood Circ. and Respiratory Flow, p. 2, 1970.

12. Bugliarello, G., Kapur, C., and Hsiao, G.: The Profile Viscosity and Other Characteristics of Blood Flow in a Non-Uniform Shear Field, Proc. 4th Int. Rheology Conf. (edited by Copley, A. L.), 4:351, 1964.
13. Brenner, H., and Bungay, P. M.: Rigid-Particle and Liquid-Droplet Models of Red Cell Motion in Capillary Tubes, Fed. Proc., 30:1565, 1971.
14. Forstrom, R. J., Blackshear, P. L., Keshaviah, P., and Dorman, F. D.: Fluid Dynamic Lysis of Red Cells, Advances in Bioengineering, Chem. Eng. Progress Symp. Series, 67:69, 1971.
15. Rand, R. P., and Burton, A. C.: Area and Volume Changes in Hemolysis of Single Erythrocytes, J. Cell. Comp. Physiol. 61:245, 1963.
16. Leonard, E. F., and Friedman, L.: Thrombogenesis on Artificial Surfaces: A Flow Reactor Problem, Chem. Eng. Prog. Symp. Ser., 66:59, 1970.
17. Petschek, H. E., and Madras, P. N.: Thrombus Formation on Artificial Surfaces. Proc. Artif. Heart Program Conf., (edited by Hegyeli, R. J.) Washington, D.C.: U.S. Govt. Printing Office, p. 271, 1969.
18. Goldsmith, H. L.: Red Cell Motions and Wall Interactions in Tube Flow. Fed. Proc., 30:1578, 1971.
19. Blackshear, P. L., Forstrom, R. J., Dorman, F. D., and Voss, G. O.: Effect of Flow on Cells Near Walls, Fed. Proc. 30:1600, 1971.

DISCUSSION

Sidney Cassin, University of Florida, Gainesville, Fla.: Is there a relationship between the size of the cell in the other species and the age of the animal? In other words, are the goat's red cell forms in fetal blood much larger than in the mature goat?

Dr. Blackshear: I think I'm the wrong person. We didn't study this in other species. Does anyone know if fetal goat blood is larger? I would expect it would be. What do you think, Bert?

Bert K. Kusserow, University of Vermont, Burlington, Vermont: I don't know about goats, but in the dog, the younger red cell forms seem to be slightly larger. The possibility may exist, too, as the cell moves out from the marrow and is released in the blood stream, that there may be a slight decrease in size. This holds true for the dog. I am not familiar with goat's fetal blood.

Dr. Cassin: Why were precautions taken to have a controlled environment and fixed plasma in all of the experiments?

Dr. Blackshear: Because it is a very sensitive thing.

John T. Reeves, University of Kentucky, Lexington, Ky.: How does the position of the tube relate to this? I presume this was in fairly large diameter vessels, but I wonder as you get to approach the capillary side, how this will influence your analysis.

Dr. Blackshear: Well, for the larger size vessels, it's pretty clear that the smaller, more rigid cells should come closer to the wall. In the work that's been done on the capillary flow, the less deformable the cell, the closer the cell goes to the wall. Hence, one would guess that capillary resistance, all things being equal, should be higher in the goat cell than it is in the elephant cell, simply because it would resist deformation. I should add that one of the consequences of this shear stress analysis is to conclude that, of the species studied, the membrane tension at lysis is the same for all mammalian species. We had never been able to demonstrate this before now.

THE EFFECTS OF THE CATECHOLAMINES AND GLUCAGON ON THE PERFORMANCE OF THE EQUINE HEART

W. C. Bowie, R. C. Purohit, R. R. Adams*, K. T. Somajah, E. W. Hawthorne and J. E. Hinds**

The equine heart because of its large size is ideally suited for studies of cardiac dynamics. Surgical techniques have been developed to implant various measuring devices in and around the left ventricle of this species. Anesthesia was achieved with thiopental sodium, and maintained with pentobarbital sodium or with halothane for the entire period of surgery. Mercury gauges were positioned on the left ventricle to record left ventricular circumference, wall thickness and upper and lower segmental lengths. From the above dimensional measurements, left ventricular volumes were calculated; additionally the internal axis ratio of the left ventricle was derived. In further studies the circumference gauge was replaced with a pair of mutual inductance coils to measure the internal diameter of the left ventricle, throughout the cardiac cycle.

By knowing the changes in radius from end-diastolic to end-systolic size of the left ventricle and the internal axis ratio, ventricular volumes were calculated. Along with measurement of changes in dimensions, ventricular aortic and arterial pressures were also measured. Information obtained as a result of the above measurements permitted us to make the following determinations: End-diastolic volume, end-systolic volume, stroke volume, cardiac output, ejection fraction, stroke work, left ventricular end-diasystolic, end-systolic and aortic pressures.

Thus in acute and chronic horses, the effects of catecholamines and glucagon were studied to evaluate their effects on the performance of the equine heart. In conscious standing instrumented horses the ventricular function studies were done by the rapid intravenous infusion of Tyrode's solution. The development of the surgical technique to implant these various transducing devices in chronic horses has permitted us to make such studies in this animal over a prolonged period of time.

INTRODUCTION

A historical review of cardiopulmonary studies in horses has been published by Garner et al.[1] and documents the contributions of this species to our knowledge of cardiovascular physiology. The large size of the cardiovascular structures in the horse makes this species a unique model for hemodynamic studies involving the simultaneous implantation of several transducing devices. Techniques for performing thoracic surgery in the horse are relatively recent[2] and closely parallel the advances in equine anesthesiology.[3-8] Such progress has made it possible for many investigators to increasingly utilize the potential offered by this species. Bowie et al.[9] have used horses to calculate the end-diastolic, end-systolic and stroke volumes from direct measurements of linear dimensional changes of the left ventricle in anesthetized animals. Eberly et al.[10,11] have used this species to evaluate cardiac output using the dye dilution technique as well as heart rate and blood pressures in awake and anesthetized animals. The relationship of ventricular volume to body weight in horses and other mammals has been studied by Holt et al.[12] These reports clearly demonstrate the usefulness of horses in cardiovascular experimentation.

Previous studies undertaken in our laboratory have been directed toward devising methods for the continuous measurement of left ventricular dimensional changes throughout the cardiac cycle[9,13] and the use of these measurements to derive estimates of chamber volume, muscle mass, mural stresses and indices of cardiac performance. The present studies were conducted to examine the hemodynamic effects of the catecholamines and glucagon in both anesthetized and conscious horses. The response of the cardiovascular system to chemical inotropic stimuli in this species and its relationship to canine and human standards in this regard has not been examined previously.

MATERIALS AND METHODS

Random source horses were quarantined and conditioned for two weeks prior to surgery. The

* School of Veterinary Medicine, Tuskegee Institute Tuskegee Institute, Alabama.

** College of Medicine, Howard University Washington, D. C.

horses were fasted 24 hours before surgery. A jugular catheter was implanted for intravenous administration of drugs. One hour before the thoracic surgery, the animals were tranquilized with propiopromazine hydrochloride (0.4 μg/kg). Surgical anesthesia was induced with thiopental sodium and maintained with either pentobarbital sodium or with halothane in all the acute experimental studies. An endotracheal tube was inserted and positive pressure respiration was maintained with a custom built large animal respirator. Halothane and nitrous oxide in combination were the maintenance anesthetics of choice in the chronic preparation.

The thoracic cavity was opened by an incision at the fifth intercostal space. For acute studies the fourth, fifth and sixth ribs were resected to expose the heart and for chronic studies only the fifth rib was resected. The rib was freed from the periosteum and then transected near the junction of the upper proximal and middle third with an obstetrical wire saw. Resection was completed by disarticulating the rib at the costo-chondral junction. The periosteum and pleura were incised to open the thoracic cavity. Hexylcaine solution (5%) was injected in the pericardial space to decrease myocardial sensitivity. In the acute preparation, mercury-in-rubber gages were implanted to measure left ventricular circumference (LVC), left ventricular wall thickness (LVWT) and two segmental lengths, upper segment length (USL) and lower segment length (LSL). These gage placement techniques in horses have been previously described.[9,13]

External ventricular volume was calculated by the method previously described by Bowie.[2] A sum of a series of segmental volumes represented the external ventricular volume. The volume of each segment may be calculated if the height and cross sectional area of the segment are known. The accuracy with which each segmental volume may be calculated and the number of segments measured determines the accuracy of this approach.

The internal volume was derived from these external dimensional calculations by subtracting the left ventricular muscle volume (MV), (obtained immediately after sacrificing the horse) from the calculated external volume.

These calculated volumes—both internal and external—were compared with the actual volumes obtained at necropsy. The hearts were fixed quickly by infusing gluteraldehyde into the left main coronary artery until cessation of heart beat occurred. The hearts were then removed and the volume of the left ventricular muscle was determined by water displacement. Casts of the horse's left ventricular cavity arrested in either diastole or systole were made, using plastic materials (Figure 1). The volume of the casts were measured by water displacement and these measurements were used to approximate the closeness of fit of the calculated volume to the actual volume.

From the above dimensional measurements the average internal end diastolic axis ratio (AR_I) was found to be 3.86. By assuming that the (AR_I) remained constant during ejection, only the end-diastolic and end-systolic internal radius of the left ventricle was needed to calculate the end-diastolic, end-systolic and stroke volume using the following equations:

$$LVEDV = 4/3\pi R^3_I \; EDV \; (AR_I) \qquad (1)$$

$$(AR_I) = 3.86$$

$$LVESV = 4/3\pi R^3_I \; ESV \; (AR_I) \qquad (2)$$

$$\text{Stroke volume (SV)} = LVEDV - LVESV$$

whereas $R_{I\ EDV}$ = internal radius of the left ventricle at the end-diastole and $R_{I\ ESV}$ = internal radius of the left ventricle at end-systole.

Cardiac output was determined by multiplying the stroke volume by the heart rate.

Those animals which were prepared for chronic cardiovascular studies were instrumented with a set of electromagnetic coils positioned across the anterior to posterior internal diameter of the left ventricle (Figure 2). The principle of operation of electromagnetic induction for motoring cardiac dimension has previously been described by Perry and Hawthorne.[14] Large bore catheters (P. E. 360) were implanted in the left ventricle and descending thoracic aorta. At the time of recording, tip mounted micromanometers were passed into the left ventricle and aorta through these catheters to record the pressures. In these animals, left ventricular volume estimates were ob-



FIGURE 1.—Plastic casts of the horse's ventricle in diastole and systole.

tained by use of equations 1 and 2 assuming a constant axis ratio of 3.86.

This report will be confined to the effects of catecholamines, (epinephrine, norepinephrine and isoproterenol) glucagon and tyrode's infusion on cardiac dynamics in the equine heart. Thirteen horses were used for epinephrine studies, sixteen for norepinephrine and fifteen for isoproterenol. Equimolar concentrations (1 mg/ml) of the drugs, epinephrine, norepinephrine, and isoproterenol were infused via the jugular vein. The rate of infusion for epinephrine and norepinephrine was 0.764 ml per minute, whereas it was 0.191 ml per minute for isoproterenol. Cardiac cycles being analysed and compared were taken at peak response time. In three conscious, standing horses, 10 mg of glucagon having a concentration of 1 mg/l ml was administered into the jugular vein. To study the effect of volume overload on the cardiac function, a rapid infusion of Tyrode's solution was given into the jugular vein.

Figure 3 illustrates the variables normally recorded in the conscious standing horse (EKG, left ventricular internal diameter, left ventricular pressure, aortic pressure and dp/dt). All variables were recorded simultaneously on an eight channel strip chart recorder and on electromagnetic tape.

RESULTS AND DISCUSSION

In acute studies, twelve horses were used for the control measurements of the cardiovascular dynamics (Table I). The data for these horses were derived from the linear dimensional measurements previously described. These horses were under the influence of an anesthetic agent for the entire period of the experiment. The mean cardiac output in the absence of the effects of the catecholamines was 22.1 $\pm$ 2.0 liters per minute which is similar to our previously reported values[9] and those of Eberly and his collaborators.[10,11] Horses anesthetized with barbiturates alone had an average cardiac output of 21.47 liters/min, while those anesthetized

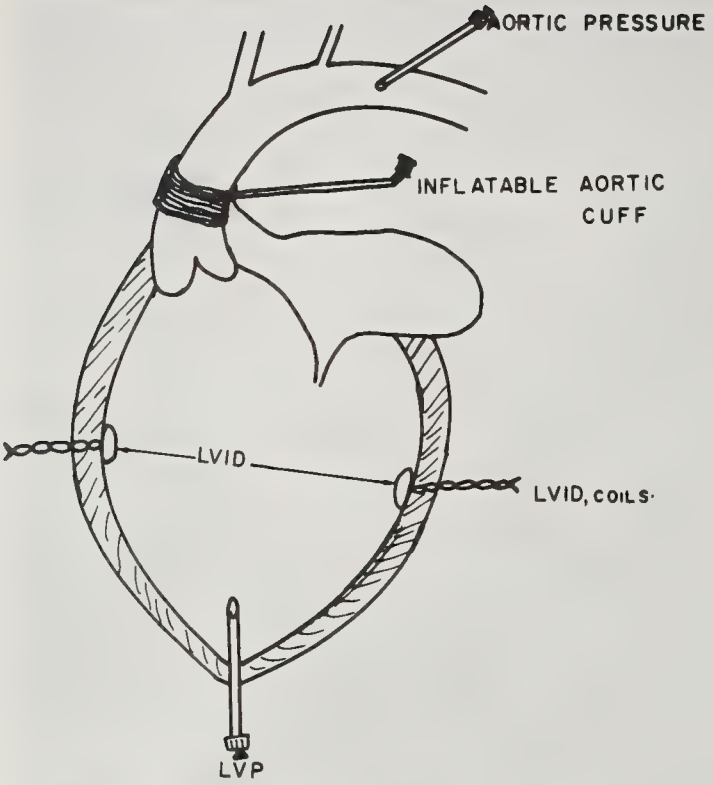


FIGURE 2.—Diagramatic representation of a pair of coils implanted in the left ventricle of the horse. LVID = Left ventricular internal diameter coils. LVP = Left ventricular pressure transducer.

with short acting barbiturates and maintained with halothane had an average cardiac output of 23.78 liters/min. The heart rates of all anesthetized animals varied from 48 to 97 beats per minute, with mean value of 72 ± 4. The increased heart rate in anesthetized horses was obvious when compared to the normal standing horses, whose mean heart rate was 40 beats per minute. Horses under the influence of pentobarbital sodium had higher heart rates than those maintained on halothane. Studies of Barlow and Knott[15] with dogs have shown that pentobarbital sodium causes a marked increase in mean arterial pressure, heart rate, cardiac output and peripheral resistance, with a decrease in average total blood volume and stroke volume. Our studies and those of Eberly and coworkers[10,11] show that the anesthetics pentobarbital and halothane both cause a marked decrease in the cardiac output, even though the heart rate is increased.

The cardiovascular values for six conscious standing horses are listed in Table II. The mean cardiac output for this group was 53.30 liters per minute. This value is slightly higher than that reported by Eberly and co-workers.[11]

Tyrode's Solution Infusion

Tyrode's solution was rapidly infused into two conscious horses in an attempt to describe the normal ventricular function curve for this species. Analysis was made of the relationship between left ventricular volume and pressure and left ventricular volume and stroke work (Figures 4 and 5). The left ventricular internal end-diastolic radius and end-systolic radius for each horse at control recording and at the maximum response are listed in Tables IIIa and IIIb along with the calculated ventricular volumes. Rapid infusion of Tyrode's solution resulted in an increase in left ventricular internal radius at end-diastole from 3.938 cm to 4.85 cm (horse #044) and from 4.4 to 4.8 cm (horse #011). Likewise, end-systolic diameter rose from 3.30 cm to 4.13 cm in horse #044 and from 3.4 cm to 3.7 cm in horse #0011.

In an attempt to evaluate the response of the

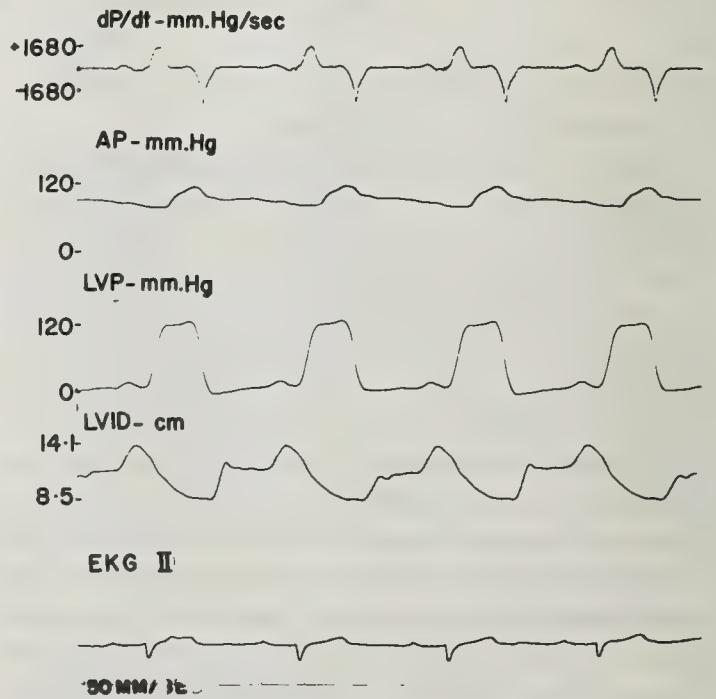


FIGURE 3.—Illustrates the parameters normally recorded in a conscious standing horse. Whereas
LVID = Left ventricular internal diameter.
LVP = Left ventricular pressure.
AP = Aortic pressure.
EKG = Lead II of the electrocardiogram.

TABLE I.—*Cardiovascular Control Measurements in Anesthetized Horses*

Horse No.	End-Diastolic Volume (ml)	End-Systolic Volume (ml)	Stroke Volume (ml)	Heart Rate (beats/min)	Cardiac Output (L/min)	Ejection Fraction (% EDV)	Stroke Work (Gm-meters)
014	782.1	413.5	368.6	97	34.7	47.2	371.2
017	1127.7	645.0	482.6	71	34.4	42.5	395.2
018	688.5	420.9	267.6	68	18.2	38.5	224.0
019	811.2	512.7	298.5	60	17.3	33.8	276.5
022	1082.1	699.1	383.0	60	23.1	34.7	327.3
0001	1114.7	816.7	298.0	86	25.9	26.7	274.0
0008	1566.5	1300.3	247.2	54	13.3	15.8	185.6
0014	1116.2	760.2	356.0	83	29.7	35.4	290.4
0015	756.2	493.5	262.5	72	18.9	34.8	223.0
0018	756.6	405.7	350.9	67	23.5	46.4	276.6
0019	831.2	479.3	298.5	60	17.2	31.8	296.4
0020	1189.0	1044.5	144.3	94	13.6	12.2	111.7
Mean	987.4	669.7	312.1	72	22.1	32.8	271.7
S.E. ±	70.5	73.9	23.2	4	2.0	2.7	21.5

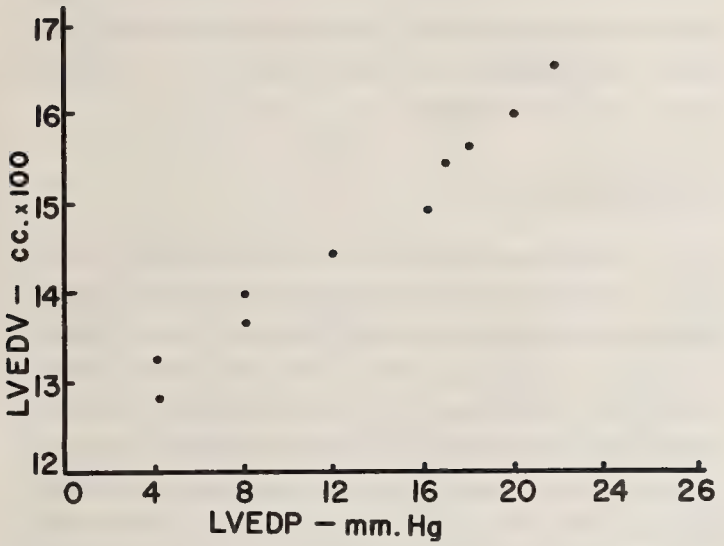


FIGURE 4.—Left ventricular pressure volume relationship due to rapid infusion of Tyrode's solution; whereas LVEDP = Left ventricular end diastolic pressure and LVEDV = Left ventricular end diastolic volume.

left ventricle the left ventricular EDV, ESV, SV, EF, SW and CO were determined prior to and during the rapid intravenous infusion of Tyrode's solution. Rapid infusion of Tyrode's solution produced increases in EDV, SV, EF, SW and CO. The increase in cardiac output was mainly duc to an increased stroke volume, since the heart rate was virtually unchanged. Bishop et al.[16] have demonstrated that in conscious dogs, the rapid infusion of Tyrode's solution, results both in increases in heart rate and stroke volume. The present study in horses suggests that the elevation in stroke volume was due to an increase in the end-diastolic size of the ventricle evoking a Frank-Starling response.

Ventricular function curves were plotted from the data derived from the rapid infusion of the Tyrode's solution. The relationship of left ventricular end-diastolic pressure (LVEDP) to left ventricular end-diastolic volume (LVEDV) is illustrated in Figure 4. The increase in end-diastolic volume was associated with a curvelinear increase in the end-diastolic pressure. The contour of the curve depicted here is similar to that which has been obtained on dogs by Tsakiris et al.[17] The relation of left ventricular end-diastolic volume and the stroke work is il-

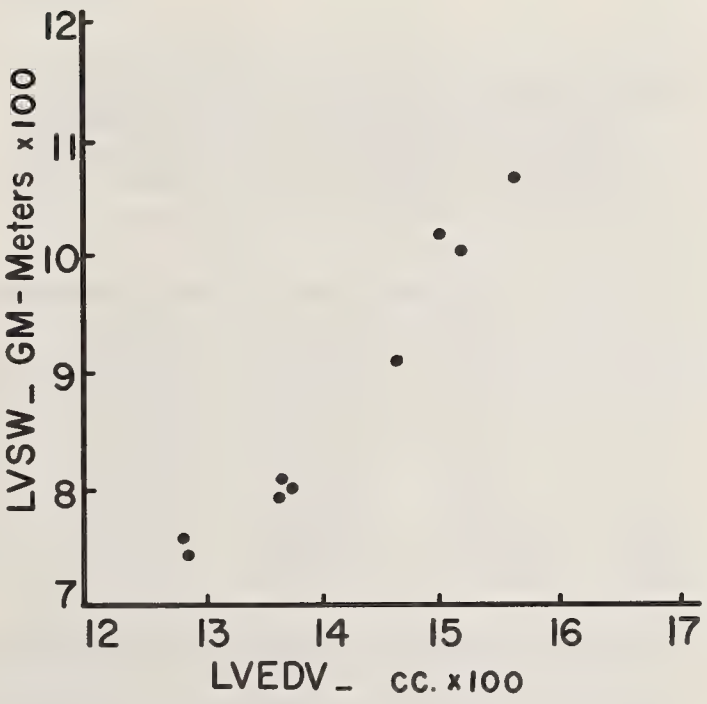


FIGURE 5.—Relationship of left ventricular end diastolic volume (LVEDV) and the left ventricular stroke work (LVSW) as a result of the rapid infusion of Tyrode's solution.

TABLE II.—*Cardiovascular Control Measurements in Normal Standing Horses*

Measurements	Horse No. 70030	70044	71003	71006	71008	71011	(Mean) (6 Horses)
End-diastolic volume (ml)	2019.7	1165.3	1985.4	1929.0	2056.3	957.9	1685.6
End-systolic volume (ml)	113.3	614.6	1318.9	822.0	1060.5	490.9	906.7
Stroke volume (ml)	889.7	550.7	666.5	1107.0	995.8	467.0	779.4
Stroke work (Gm-meters)	895.3	—	—	1084.0	1173.7	508.1	—
Heart rate (beats/min)	54.0	80.0	66.0	75.0	59.0	90.0	70.6
Cardiac output (L/min)	48.0	44.0	43.9	83.0	58.7	42.0	53.3
Ejection fraction (% EDV)	44.0	47.0	—	57.4	48.4	48.8	—

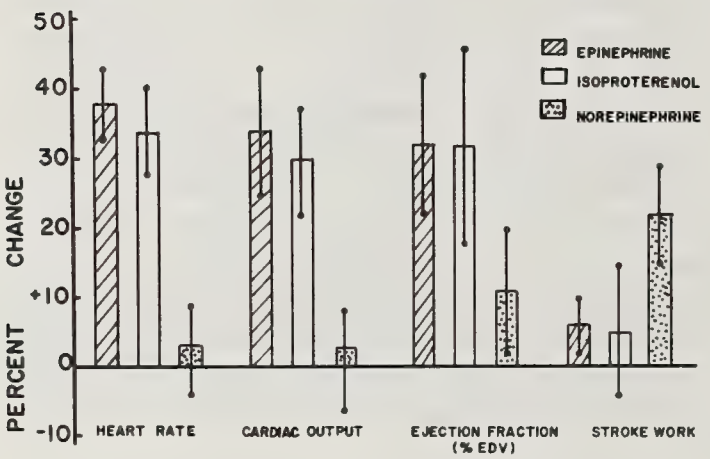


FIGURE 6.—Comparison of the percent change in cardiovascular values after intravenous administration of Epinephrine, Isoproterenol and Norepinephrine. Bars represent the mean of 13 values and brackets represents the standard errors of these means.

lustrated in Figure 5. The direct correlation between increased EDV resulting in increased stroke work is apparent.

Catecholamines

The effects of continuous intravenous infusion of epinephrine, norepinephrine and isoproterenol are summarized in Table IV and Figure 6. Horses used in these studies were either under the influence of pentobarbital sodium anesthesia for the entire period of the experiment or the anesthesia was induced by pentobarbital sodium and maintained on halothane.

The intravenous infusion of epinephrine caused a marked increase in the heart rate, the cardiac output, the ejection fraction and a decrease in the left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV)

TABLE III$_a$.—*Effects of Tyrode's Solution Infusion on Cardiovascular Measurements in a Chronic Horse (#0011)*

End-diastolic Radius (cm)	End-systolic Radius (cm)	End-diastolic Volume (ml)	End-systolic Volume (ml)	Stroke Volume (ml)	Ejection Fraction (% EDV)	Heart Rate (beats/min)	Cardiac Output (L/min)	Stroke Work (Gm-meters)
4.40	3.40	1275.0	588.3	686.7	53.85	86	58.86	722.0
4.50	3.50	1363.4	641.7	721.7	52.93	83	59.90	798.2
4.60	3.50	1456.9	641.7	815.2	55.93	80	65.21	916.4
4.65	3.50	1504.9	641.7	863.2	57.35	79	68.19	1025.2
4.70	3.55	1554.0	669.6	884.4	56.90	81	71.63	1074.4
4.80	3.60	1655.3	698.3	957.0	57.81	82	78.47	1310.2
4.80	3.70	1655.3	758.2	897.1	54.19	83	74.49	1285.7

TABLE III$_b$.—*Effect of Tyrode's Solution Infusion on Cardiovascular Measurements in a Chronic Horse (#044)*

End-Diastolic Radius (cm)	End-systolic Radius (cm)	End-diastolic Volume (ml)	End-systolic Volume (ml)	Stroke Volume (ml)	Ejection Fraction (% EDV)	Heart Rate (beats/min)	Cardiac Output (L/min)
3.93	3.30	908.5	537.6	370.6	40.79	86	31.9
4.12	3.50	1050.6	641.7	408.8	38.90	80	32.7
4.50	3.45	1363.9	614.6	749.3	54.93	86	64.4
4.60	3.45	1456.9	614.6	842.3	57.81	86	72.4
4.85	4.125	1707.6	1050.6	657.0	38.47	86	56.5

TABLE IV.—*Cardiovascular Measurements in Anesthetized Horses (Effect of Catecholamines)**

Treatment	End-diastolic Volume (ml)	End-systolic Volume (ml)	Stroke Volume (ml)	Heart Rate (beats/min)	Cardiac Output (L/min)	Ejection Fraction (% EDV)	Stroke Work (Gm-meters)
Control	987.4 ± 70	669.7 ± 74	312.1 ± 23.2	72 ± 6	22.1 ± 2	32.8 ± 2.7	271.7 ± 21.5
Epinephrine	862.5 ± 95	562.7 ± 92	303.7 ± 32	99 ± 6	29.6 ± 3	42.8 ± 4.6	285.8 ± 9.3
Control	1056.4 ± 88	425.9 ± 80	320.4 ± 25.6	68 ± 4	21.6 ± 2	31.7 ± 2.6	276.5 ± 26.7
Isoproterenol	886.8 ± 73	575.7 ± 83	313.5 ± 43.3	91 ± 5	27.0 ± 3	41.5 ± 5.0	288.6 ± 37.5
Control	1032.8 ± 85	712.6 ± 76	316.8 ± 26.2	68 ± 6	20.0 ± 2	31.9 ± 2.3	271.3 ± 23.3
Norepinephrine	908.0 ± 93	621.6 ± 80	286.7 ± 30.3	70 ± 5	20.5 ± 2	35.5 ± 3.1	331.6 ± 39.6

* Number of Horses used in each study are: Epinephrine 13, Isoproterenol 15 and Norepinephrine 16.

without a significant change in stroke volume and stroke work. The mean increase in cardiac output was 7.5 liters/min which represented a 33.94% increment. The left ventricular end-diastolic volume for the thirteen control horses ranged from 688.5 to 1,566.5 ml (mean EDV = 987.4 ± 70 ml) ; whereas in the animals infused with epinephrine, the range was 413.5 to 1,529.0 ml (mean EDV = 826.5 ± 95 ml). The end-systolic volume was similarly higher in the control horses (mean ESV = 669.7 ± 74 ml) than in the epinephrine infused animals (mean ESV = 562 ± 92 ml). The left ventricular stroke work was slightly increased from control mean values of 271.5 ± 21.5 gm M/beat to 285.8 ± 29.3 gm M/beat. The increment in the ejected fraction was 30.5% (from 32.8 ± 2.7 to 42.8 ± 4.6). Thus both an increase in the ejection fraction and the heart rate (37.5%) resulted in more complete systolic emptying.

Isoproternol consistently produced similar directional changes in hemodynamics as did epinephrine. The mean left ventricular end-diastolic volume decreased from 1052.4 ± 88.5 ml to 886.8 ± 73.3 ml. A similar decrease in end-systolic volume (from 725.9 ± 80 to 575.7 ± 83.7 ml) was seen. Stroke volume was not altered in any consistent fashion. The mean increase in heart rate was 33.8% while the mean increase in cardiac output was 25.00%. The cardiac output was increased primarily through an increase in heart rate, while stroke volume generally remained unchanged.

The effects of norepinephrine differed markedly from those of epinephrine and isoproterenol. The heart rate and cardiac output were not appreciably changed (Table IV). A slight decrease in both the end-diastolic and the end-systolic volumes was noted. Stroke volume was not increased above the control value. There was a slight increase in the ejection fraction from 31.9 ± 2.3% to 35.5 ± 31%, but these increments were not consistent from one animal to another. There was a marked increase in the calculated stroke work index (22.15%).

The action of epinephrine and isoproterenol on the equine heart consistantly resulted in an increased cardiac output, an increased heart rate, a decrease in end-diastolic volume, a decrease in end-systolic volume, and an increase in the ejection fraction, with no appreciable change in either stroke volume or stroke work. Whereas, norepinephrine caused a slight decrease in the end-diastolic volume, a decrease in the end-systolic volume but had no significant effect on heart rate, cardiac output or stroke volume. The ejection fraction was slightly increased and a marked increase in stroke work occurred.

In our studies with horses, the major effect of epinephrine and isoproterenol was to increase the cardiac output by increasing the heart rate. James and Nadeau[18] have shown that the increased heart rate produced by isoproterenol is due to a direct action of this drug and its analogs on the sinus node. The infusion of epinephrine and isoproterenol caused a decrease in the end-diastolic and end-systolic volumes along with an unchanged or slightly decreased stroke volume. This more complete ventricular emptying contributes in part to the elevation of cardiac output. The increased cardiac output seen in horses as the result of epinephrine infusion was 33.94% above the control value; whereas, in normal human subjects, the maximum rise in the cardiac output response is approximately 50 percent larger than control values.[19] Several studies have demonstrated that the cardiac output is consistently increased following the administration of epinephrine regardless of the species (man, dog, cat, rabbit, etc.).[20-24] Dodge,[25] while studying the effects of isopro-

terenol on human subjects, observed an increased cardiac output, stroke volume and stroke work of both left and right ventricles. The increased stroke volume due to the administration of isoproterenol noted in humans was not consistently found in horses. We have observed a slightly decreased or unchanged stroke volume most frequently, while the stroke work was unaltered or slightly increased.

The effects of epinephrine and isoproterenol were quite different from those of norepinephrine. The latter drug caused an increase in stroke work without any significant change in cardiac output or heart rate.

The intravenous infusion of norepinephrine usually reduces the cardiac output in man. Originally, this was shown by Goldenberg and coworkers.[21] Barcroft and Starr[19] have shown similar effects in man using ballistocardiography. On the other hand, Frank et al.,[26] using the same method, noted an increase in cardiac output in response to norepinephrine infusion prior to bleeding but not during hemorrhagic shock in the dog. Using a dye dilution technique to measure the cardiac output in the dog, Gilmore et al.[27] arrived at a contrary conclusion. It was apparent in our studies that no significant change occurred in cardiac output with norepinephrine, even though the left ventricular contractility was increased significantly when judged by the marked increase in stroke work which occurred from a smaller end-diastolic size.

Glucagon

The positive inotropic and moderate chronotropic effect of glucagon in normal cardiac muscle has been clearly established in various animal preparations[28–32,36] and in man.[33–35,37] Studies were undertaken in this laboratory to evaluate the cardiotonic effects of glucagon in horses. Only conscious horses were used in these studies.

Intravenous administration of glucagon in awake horses resulted in increased left ventricular end-diastolic volume, increased stroke volume, increased heart rate, increased stroke work, increased ejection fraction, a slight increase in left ventricular peak pressure and a virtually unchanged left ventricular end-systolic volume (Tables V and VI). The mean left ventricular end-diastolic volume (EDV) increased for the three horses studied from 1,635 to 1,977 ml. The increase in EDV in the absence of a significant change in ESV was reflected in an increased stroke volume (from a mean value of 821.33 to 1149.8 ml). The increases in heart rate and stroke volume resulted in an increase in the cardiac output (from a mean value of 57.69 to 90.80 lit/min). There was also an increase in stroke work (from 829.13 to 1190.60 gm M) and ejection fraction (from 50.08 to 59.64%).

Kumar et al.[38] reported increased heart rates and cardiac output in dogs from glucagon infusions, but stroke volume decreased. In the present study the stroke volume was increased and the resultant increase in cardiac output was due to both an increase in heart rate and stroke volume. In human studies, it has been shown that following glucagon infusion the increase in cardiac output was due more to an increase in stroke volume than an increase in heart rate.[39]

In the present study with horses, the onset of glucagon action was rapid with significant effects seen within 1 to 2 minutes after intravenous administration of the drugs. However, the

TABLE V.—*Effect of Glucagon Infusion on Cardiovascular Measurements in Chronic Horses*

Horse No.	Treatment	End-Diastolic Volume (ml)	End-systolic Volume (ml)	Stroke Volume (ml)	Heart Rate (beats/min)	Cardiac Output (L/min)	Ejection Fraction (% EDV)	Stroke Work (Gm-meters)	Left Ventricular Pressure PSP
030	Control	2019.7	1133.3	889.7	54	48.04	44.05	895.3	126
	Glucagon	2791.0	1433.3	1358.0	63	76.00	48.70	1274.3	132
006	Control	1929.	822.	1107.	75	83.0	57.4	1084.	116
	Glucagon	2109.	642.	1464.	88	128.8	69.5	1592.	124
0011	Control	957.9	490.9	467.0	90	42.03	48.80	508.1	124
	Glucagon	1031.6	404.1	627.5	108	67.77	60.82	705.5	128
Mean	Control	1635.53	815.4	821.23	73	57.69	50.08	829.13	122
	Glucagon	1977.20	826.8	1149.80	86.3	90.86	59.67	1190.60	128

TABLE VI.—*Effect of Glucagon Infusion on Cardiovascular Measurements in a Chronic Horse (10 mg)*

Time Min	Time Sec	End-Diastolic Volume (ml)	End-systolic Volume (ml)	Stroke Volume (ml)	Heart Rate (beats/min)	Cardiac Output (L/min)	Ejection Fraction (% EDV)	Stroke work (Gm-meters)	Left Ventricular Pressure	Aortic Pressure
									PSP	PSP
0		957.9	490.9	467.0	90	42.0	48.8	508.1	124	108
1		1031.6	445.9	585.7	102	59.7	56.7	647.8	120	104
2		1031.6	404.1	627.5	107	67.1	60.8	716.8	124	108
2	30	1031.6	445.9	585.7	111	65.0	56.7	669.1	128	112
2	45	1031.6	404.1	627.5	108	67.7	60.8	705.5	128	118
3		1031.6	445.9	585.7	108	61.5	56.7	690.3	124	108
4		957.9	404.1	627.5	105	58.1	57.8	582.4	120	100
5		957.9	449.9	508.0	106	53.8	53.0	534.0	120	96
7		1031.6	445.9	585.7	105	61.5	56.7	690.3	128	108
8		1031.6	490.5	541.1	102	55.1	52.4	773.4	132	118
9		1190.0	537.8	652.1	94	61.3	54.7	827.7	138	124
10		957.9	445.9	512.0	97	49.6	53.4	547.7	120	104
11		957.9	445.9	512.0	100	51.2	53.4	570.9	126	104
12		1108.9	490.	618.0	97	59.9	55.7	706.	128	104
15		957.9	445.9	512.0	95	48.5	53.4	529.2	116	84

cardiovascular response reached a peak 2 to 3 minutes later and was followed by a gradual decrease in activity. There was not a complete return to the control values after 15–20 minutes.

The effect of glucagon infusions on the hemodynamic system of the horse appears to be more akin to those reported in man[39] than to the responses found in dogs. These effects are characterized by an increase in the cardiac output resulting equally from elevations in heart rate and stroke volume. In dogs, the increase in cardiac output is due entirely to tachycardia.[38]

SUMMARY

The technique used for dimensional measurements allowed us to calculate left ventricular internal volumes during continuous cardiac cycles. Along with measurements of change in internal diameter from end-diastolic to end-systolic size, ventricular and aortic pressures were also measured. These measurements permitted us to make the following determinations: left ventricular internal end-diastolic volume, end-systolic volume, stroke volume, cardiac output, ejection fraction, and stroke work.

Studies with anesthestized and conscious horses showed that there was a marked effect of anesthesia on the cardiovascular values. Pentobarbital and halothane caused a 50% reduction in the cardiac output.

Rapid intravenous infusion of Tyrode's solution caused an increase in end-diastolic volume, end-systolic volume, stroke volume, stroke work, and the cardiac output. The increase in cardiac output was mainly due to the increased stroke volume since the heart rate was not changed. The elevation in stroke volume obtained by the volume overload was primarily due to the increase in the end-diastolic size of the left ventricle.

Isoproterenol and epinephrine produced similar directional changes in cardiovascular data of the equine heart. The effects of norepinephrine differed markedly from those of epinephrine and isoproterenol. Isoproterenol and epinephrine caused an increase in heart rate and cardiac output, and a decrease in end-diastolic and end-systolic volume, with a generally variable or unchanged stroke volume and stroke work. Norepinephrine caused a slight decrease in end-diastolic volume and end-systolic volume, unchanged or slightly variable heart rates, virtually unchanged cardiac output, variable or unchanged stroke volume and ejection fractions, and a marked increase in stroke work.

Intravenous administration of glucagon resulted in increased left ventricular end-diastolic volume, increased stroke volume, increased heart rate, and increased stroke work. Increased cardiac output was the result of both an increase in stroke volume and heart rate. This is in contrast to the response to epinephrine and isoproterenol where the increased cardiac output was the result of an increasing heart rate, but not an increasing stroke volume.

The cardiovascular effects of glucagon are similar in some respects to those of beta reactive catecholamines, these effects being both

positive inotropic and chronotropic on the equine heart.

REFERENCES

1. Garner, H. E., Rosborough, J. P., Amend, J. F., and Hoff, H. E. The grade pony as a new laboratory model in cardiopulmonary physiology. Cardiovascular Research Cent. Bull. (Published by Baylor College of Medicine), 9:91–104, (1971).
2. Fowler, M. E., Crenshaw, G. L., Edwards, D. W., Holloway, C. P., and Whatley, J. L. Intrathoracic surgery in the horse. Am. J. Vet. Res. 24 766–771, 1963.
3. Fowler, M. E., Parker, E. E., McLaughlin, R. F., and Tyler, W. S. An inhalation anesthetic apparatus for large animals. J.A.V.M.A. 143:272–276, 1963.
4. Grable, A. A., Hamlin, R., and Smith, C. R. Effects of promazine and chloral hydrate on the cardiovascular system of the horse. Am. J. Vet. Res. 25:1151, 1964.
5. Tevik, A., Sharpe, J., Nelson, A. W., Bartley, W. E., and Lumb, W. V. Effect of nitrogen in closed-circle system with low oxygen flow for equine anesthesia. J.A.V.M.A., 154:166, 1969.
6. Tevik, A., Melson, A. W. and Lumb, W. V. Chloroform and halothane anesthesia in horses: Effect on blood electrolytes and acid-base balance. Am. J. Vet. Res. 29:1791–1798, 1968.
7. Wolf, W. A., Lumb, W. V., and Ramsey, B. S. Comparison of halothane and chloroform anesthesia in horses. Am. J. Vet. Res. 29:125, 1968.
8. Grabel, A. A. The effects of intravenous ether anesthesia on the blood of equine animals. Am. J. Vet. Res. 24:993–996, 1963.
9. Bowie, W. C., Hawthorne, E. W., Adams, R. R., and Somaiah, K. T. Instantaneous changes of left ventricular volume in anesthetized horses. Am. J. Vet. Res. 32:1533–1542, 1971.
10. Eberly, P. E., Gillespie, J. R., and Tyler, W. S. Cardiovascular parameters in the thoroughbred horses. Am. J. Vet. Res. 25: 1712–1715, 1964.
11. Eberly, P. E., Gillespie, J. R., Tyler, W. S., and Fowler, M. E. Cardiovascular values in the horses during halothane anesthesia. Am. J. Vet. Res. 29 (1968) :305–314, 1968.
12. Holt, J. P., Rhode, E. A., and Kines, J.: Ventricular volumes and body weight in mammals. Am. J. Physiol. 215: 704–715, 1968.
13. Cothran, L. N., Bowie, W. C., Hinds, J. E., and Hawthorne, E. W. Left ventricular wall thickness changes in unanesthetized horses. In: *Factors Influencing Myocardial Contractility*. Edited by R. D. Tanz, F. Kaveler and J. Roberts. Academic Press, New York, N. Y., pp. 163–176, 1967.
14. Perry, S. L. C., and Hawthorne, E. W. Left ventricular volume as measured by electromagnetic plethysmography. J. Natl. Med. Assoc. 50:11M, 1958.
15. Barlow, G., and Knott, D. H. Hemodynamic alteration after 30 minutes of pentobarbital sodium anesthesia in dog. Am. J. Physiol. 207:764–766, 1964.
16. Bishop, V. S., Horwitz, L. D., Stone, H. L., Stegall, H. F., and Engelken, E. J. Left ventricular internal diameter and cardiac function in conscious dogs. J. Appl. Physiol. 27:619–623, 1969.
17. Tsakiris, A. F., Vandenburg, R. A., Banchero, N., Sturm, R. E., and Wood, E. H. Variations in left ventricular end diastolic pressure, volume, and ejection with changes in outflow resistance in anesthetized intact dog. Cir. Res. 23:213–222, 1968.
18. James, T. N., and Nadeau, R. A. Vagal-blacking action of two analogs of isoproterenol studied by direct perfusion of the sinus node. Clin. Res. 11:169, 1963.
19. Barcroft, H., and Starr, I. Comparison of action of adrenaline and noradrenaline on cardiac output in man. Clin. Sci. 10:295, 1950.
20. Domingo, M. A. Cardiovascular effects of some commonly used pressor amines. Anesthesiology 20: 71–97, 1959.
21. Goldenberg, M., Pines, K. L., Baldwin, E. de F., Greene, D. G., and Rob, D. E. The hemodynamic response of man to norepinephrine and epinephrine and its relation to the problem of hypertension. Am. J. Med. 5:792, 1948.
22. Krasnow, N., Rolett, E. L., Yurchak, P. M., Hood, W. D., and Gorlin, R. Isoproterenol and cardiovascular performance. Am. J. Med. 37:514–525, 1964.
23. Rushmer, R. F., and West, T. C. Role of autonomic hormones on left ventricular performance continuously analyzed by electronic computer. Cir. Res. 5:240–246, 1957.
24. Sanmarco, M. E. Effects of isoproterenol and epinephrine on left ventricular volume and force-velocity relation. (Published by Am. Heart Association). A Summary of the Proc. of a Summer Workshop. July 24, 1964.
25. Dodge, H. T., Lord, J. D., and Sandler, H. Cardiovascular effects of isoproterenol in normal subjects with congestive heart failure. Am. Heart J., 60:64–105, 1960.
26. Frank, E. D., Frank, H. A., Jacob, S., Weizel, H. A. E., Korman, H., and Fine, J. Effect of Norepinephrine on Circulation of Dog in Hemorrhagic Shock. Am. J. Physiol. 186: 74–78, 1956.
27. Gilmore, J. P., Smythe, C. M., and Handford, S. W. Effect of L-norepinephrine on cardiac output in anesthetized dog during graded hemorrhage. J. Clin. Invest. 33:884, 1954.
28. Farah, A., and Tuttle, R. Studies on Pharmacology of glucagon. J. Pharm. & Esper. Therap., 129:49–55, 1960.

29. WHITEHOUSE, F. W., and JAMES, T. N. Chronotropic action of glucagon on sinus nobe. Proc. Soc. Esp. Biol. Med. 122:823, 1966.
30. GLICK, G., PARMLEY, W. W., WECHSTER, A. S., and SONNENBLICK, E. H. Glucagon: Its enhancement of cardiac performance in the cat and dog and persistance of its inotropic action despite beta-recepton blockade with propranolol. Circ. Res. 22:789–799, 1968.
31. REGAN, T. H., LEHAN, P. Y., HENNEMAN, D. M., BEHAR, A., and MELLEMS, H. K. Myocardial metabolic and contractile response to glucagon and epinephrine. J. Lab. Clin. Med. 63:638–647, 1964.
32. SUTHERLAND, E. W., and RALL, T. W. The relation of adenosine—3′5′—phosphate and phoxpherylase to the action of catecholamines and other hormones. Pharmacol. Res. 12:265, 1960.
33. PARMLEY, W. W., GLICK, G., and SONNENBLICK, E. H. Cardiovascular effect of glucagon in man. New England J. Med. 279:12–17, 1968.
34. LINHART, J. W., BAROLD, S. S., COHEN, L. S., HILDNERS, F. J., and SAMET, P. Cardiovascular effect of glucagon in man. Am. J. Cardiol. 22:706–710, 1968.
35. PARMLEY, W. W., and SONNENBLICK, E. H. Glucagon: A new agent in cardiac therapy. Am. J. Cardiol. 27:298–303, 1967.
36. ENTMAN, M. L., LEVY, G. S., and EPSTEIN, S. E. Mechanism of action of epinephrine and glucagon on the canine heart. Cir. Res. 25:429–438, 1969.
37. PURI, P. S., and BING, R. J. Effects of glucagon on myocardial contractility and hemodynamics in acute experimental myocardial infarction. Basis for its possible use in cardiogenic shock. Am. Heart J. 78:660–668, 1969.
38. KUMAR, R., SHARMA, G.V.R.K., MOLOKHIA, F. A., NORMAN, J. C., INAMDAR, A. N., MESSER, J. V., ABELMANN, W. H., and HOOD, W. B. Experimental myocardial infarction X. Efficiency of glucagon in acute and healing phase in intact conscious dogs. Circulation 45:55–64, 1972.
39. DIAMOND, G., FORRESTER, J., DANIZIG, R., PARMLEY, W. W., and SWAN, H.J.C. Acute myocardial infarction in man. Comparative hemodynamic effects of norepinephrine and glucagon. Am. J. Cardiol. 27:612–616, 1971.

DISCUSSION

C. GRANT LAFARGE, Children's Hospital Medical Center, Boston, Massachusetts: I think this paper and the one earlier by Dr. Cothran are both extremely interesting. Have you had a chance to look at any abnormal animals that you've made, either with infarction techniques or hypertrophy, or any other state. I wonder if Dr. Cothran might respond to that, too?

DR. COTHRAN: No.

DANIEL B. JACKSON, Abbott Laboratories, North Chicago, Illinois: Did you have a chance to do any studies on the relationship between the stroke volume and end-diastolic volume which is dependent on varying heart rates?

DR. PUROHIT: No, but by infusion of the Tyrode's solution, we were not able to change the heart rates. We were only able to change the end-diastolic volume by volume load, and this caused an increase in the stroke volume. No, we haven't tested these hearts to cause the heart rate to increase or decrease, which we must plan to do in the future.

AN ANALYSIS OF LEFT VENTRICULAR DIMENSIONAL CHANGES IN CONSCIOUS ANIMALS

L. N. Cothran, E. W. Hawthorne and H. Sandler*

Experiments were designed and conducted to study the continuous changes in left ventricular external and internal geometry throughout the cardiac cycle in conscious animals. An analysis of the data obtained from typical, normal cardiac cycles revealed an average decrease in the external diameter of the left ventricle of 3.5%, a 32% increase in wall thickness, an 18% decrease in the internal diameter and a 6% decrease in length during the ejection phase. These dimensional changes resulted in a 14.6% increase in the internal major to minor axis ratio (AR) of the left ventricle. A linear relation was found when the instantaneous internal radius of the left ventricle was plotted against the simultaneously derived axis ratio. The equation describing the latter relation was determined to be: Axis ratio $= -0.6\ Ri + 3$. By use of this equation to determine (AR) and the equation for the volume of a prolate ellipsoid ($V = 4/3\pi Ri^3 AR$) a method was derived for calculation of the instantaneous internal volume of the left ventricle during the ejection period from a knowledge of the internal radius alone.

INTRODUCTION

All of the methods presently available for assessing the performance of the intact heart require either direct information about the size, shape and cyclic changes in these variables or the use of certain simplifying assumptions based in part upon an a priori knowledge of dynamic cardiac dimensions. The size and shape of the left ventricle greatly affects its performance as a pump and are therefore necessary measurements for evaluation of its hydraulic functions. Additionally, in order to obtain information which would provide for characterization of the material properties and basic muscle mechanics of the left ventricle, dimensional measurements are essential. Knowledge of the importance of heart size and shape and the application of these variables in determining cardiac function is not new. These concepts are embodied in the application of the law of Laplace[1] to the ventricle by Wood[2] and in the Frank-Starling[3] mechanism. Therefore, the history of cardiac dimensional analysis covers almost one century. However, the availability of instrumentation and the techniques for measurement of individual chamber dimensions in both humans and experimental animals are relatively recent. Through the use of such techniques, it has been established that marked differences in cardiac size and performance become manifest when experimental animals are subjected to anesthetization and thoracotomy.[4] Therefore, it has been emphasized that the most meaningful way of determining the mechanisms operative in the moment-to-moment control of ventricular functions is to obtain an accurate estimate of the performance of the heart in intact, unanesthetized animals.[5]

The application of linear dimensional measurements of the left ventricle to estimates of internal volume requires the development of geometric analogs to represent the ventricular chamber. A number of geometric models of the left ventricle have been proposed and used to derive estimates of chamber volume, muscle mass and the magnitude and distribution of forces within the wall. The derivation of equations for calculating these variables is dependent upon the geometric model which is employed. The models most frequently used for these purposes have been a sphere, cylinder, cone, paraboloid and the ellipse of revolution. Dodge et al.[6] have determined the ratio of major-to-minor axis of the human left ventricle at end-diastole and end-systole and suggested the simplest geometric shape equivalent to that of the left ventricle to be a prollate ellipsoid. This observation has been supported by Hawthorne[7] from studies of the external left ventricular dimensions of dogs and by Gorlin[8] who used the same data corrected for wall thickness to obtain

* Howard University College of Medicine, Washington, D. C. and NASA-Ames Research Center, Moffett Field, California.

an internal axis ratio greater than unity. The use of an ellipse of revolution as a geometric analog of the left ventricular chamber introduces the requirement for simultaneous measurements across two perpendicular axes in order to calculate volume from linear dimensions. Of the linear dimensions required for the derivation of the volume of an ellipsoid, the measurement of length has proved to be the most difficult to obtain by analog techniques.[9]

This study deals with the measurement of transverse and longitudinal dimensions of the left ventricle using a combination of analog and cineradiographic techniques. From the linear measurements which were made, the cyclic variations in several dimensions and wall stresses were derived. In addition, an opportunity was provided to examine the cyclic changes that occur in the internal axis ratio of the left ventricle and to use this variable in estimates of chamber volume.

METHODS

Only conditioned, adult, male dogs were selected for use in these studies. The body weights of these animals ranged from 28 to 35 kilograms. Final preoperative preparation of these animals consisted of a 12-hour fast period and administration of a broad-spectrum antibiotic. Pentobarbital sodium was used to achieve a surgical plane of anesthesia. A left, anteriolateral thoracotomy was performed at the level of the fifth costal interspace. Respiration was maintained with a 40 per cent oxygen, 60 per cent air mixture administered through an endotracheal tube attached to a positive pressure respirator which was activated by a demand type valve.

Three methods were used for measurement of the transverse dimensions. In three of the ten dogs studied, the left ventricle was instrumented with a mercury-in-rubber strain gage for monitoring the external circumference. The gage was positioned around the equator of the ventricle. A set of disc-shaped coils were implanted coaxially across the anterior wall to monitor the changes in wall thickness (Figure 1).[10] In two dogs, external circumference of the left ventricle was measured with a mercury-in-rubber gage and internal diameter was measured by means of a sonomicrometer system (Figure 2).[11] In the five remaining animals, external diameter and wall thickness were measured by means of set of three coils implanted in a straight line across the left ventricle (Figure 3). In this group radiopaque markers were implanted on the left ventricle at the bifurcation of the left main coronary and at the apical dimple. These markers were used to monitor the changes in length of the left ventricle utilizing the biplane cineradiographic technique previously described by Mitchell et al.[12] In six of the dogs used in this study pressure cells were implanted in the left ventricle through the apical dimple and in the thorocic aorta two to three centimeters distal to the arch. Left ventric-

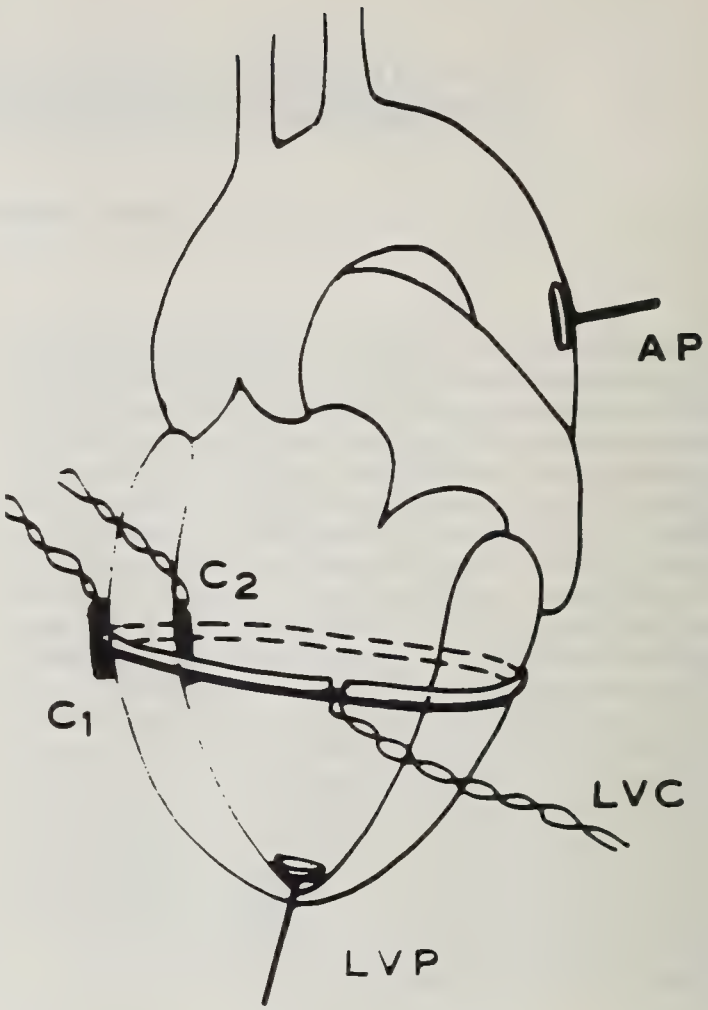


FIGURE 1.—A schematic representation of the left ventrical showing transducer placement (type I). (LVC) left ventricular circumference gage (C_1—C_2) left ventricular wall thickness coils, (LVP) left ventricular pressure, (AP) aortic pressure.

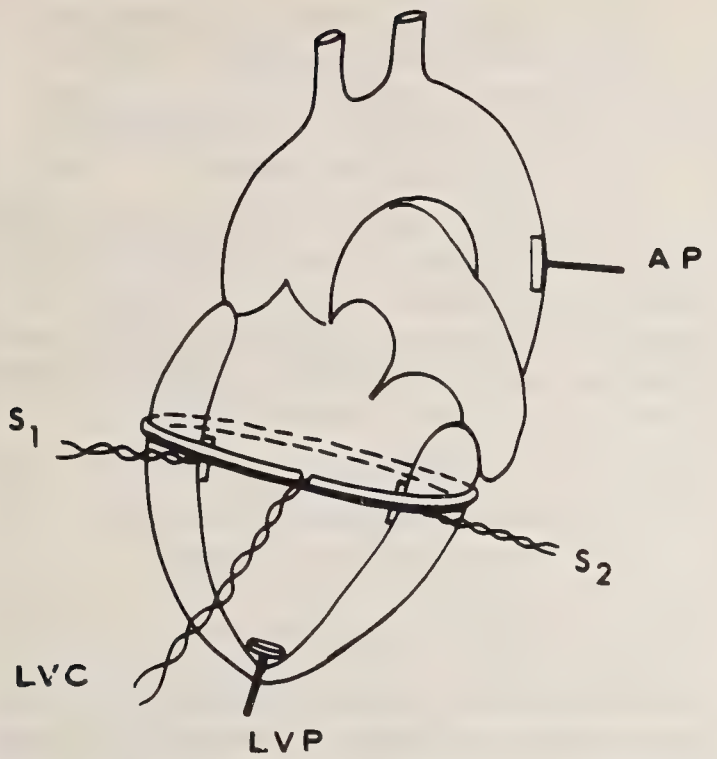


FIGURE 2.—A schematic representation of the left ventricle showing transducer placement (type II). (LVC) left ventricular circumference, (S_1—S_2) sonomicrometer for internal diameter, (LVP) left ventricular pressure, (AP) aortic pressure.

ular and aortic pressures in the remaining animals were obtained through polyvinal catheters inserted into the apical dimple and the descending aorta.

After surgery, a recovery period of seven to ten days was allowed. Records were obtained on each animal while quietly standing in a modified Pavlov sling. The five dogs studied by biplane cineradiography were lightly tranquilized and positioned in sternal recumbency in a fiber glass molded cradle and straped in place on the x-ray table. All primary variables and their calibrations were recorded on a multichannel direct writing recorder and on analog tape.

Data Reduction

The output of the magnetic tape playback was amplified, offset where necessary and input to the analog to digital converter module of a 2116B Hewlett Packard digital computer system. Selected samples of the analog signals were obtained and processed on command by instructions input through the teletype keyboard. Calibration factors were input at the start of the data reduction routine so that conversion from volts to the appropriate units of dimension was complete prior to storage of the prime data. Sampling frequency for the data presented here was set at one point every 10 milliseconds for each variable digitized for a total of 200 points per channel. The prime data was stored in a buffer array that was dimensioned by the source program. The derived data was obtained by subjecting the elements of the buffer array to the various algorithms provided in the source program. A permanent record of all data was provided by teletype printout.

The films obtained during biplane cineographic studies were digitized by hand in a frame-by-frame fashion. Each frame of the film contained in one corner, a trace of the simultaneously recorded analog data and one channel of the analog data contained a frame marker which corresponded on a one-to-one basis with

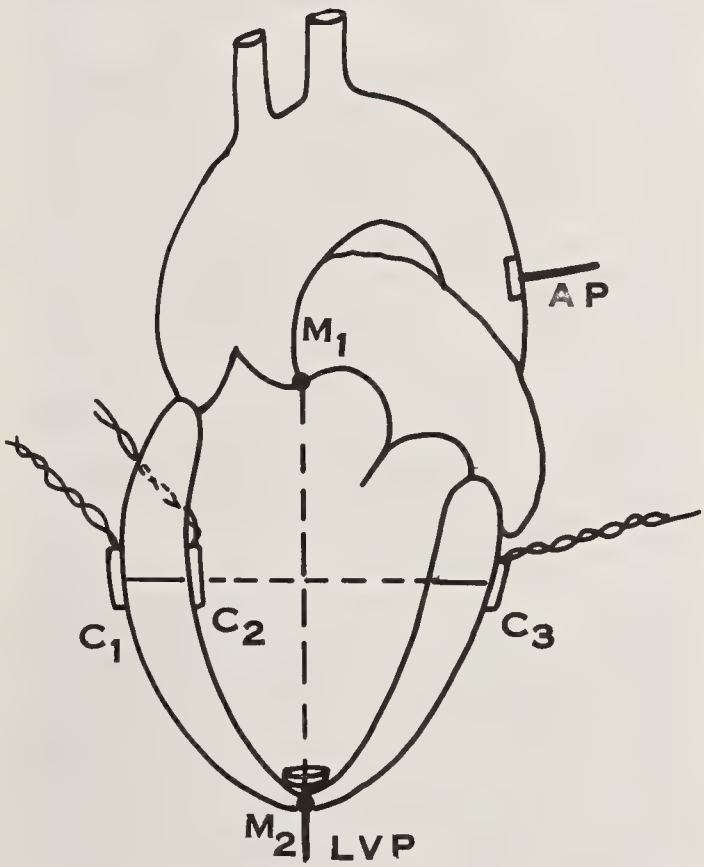


FIGURE 3.—A schematic representation of the left ventricle showing transducer placesment (type III). (C_1—C_3) external diameter coils, (M_1—M_2(radiopaque length markers, (LVP) left ventricular pressure, (AP) aortic pressure.

each frame of the film. These markers enabled the precise synchronization of the analog data with the appropriate frame of the film. Selected sections of both the dorso-ventral and the corresponding lateral cineogram were digitized. The end of each roll of film contained a calibration grid which was used to correct for geometric and spherical distortion of the digitized data.

Calculations

A) Calculation of spatial distance between two points on biplane film.

$$D = \sqrt{(X_2 + X_1)^2 - (Y_2 + Y_1)^2 - (Z_2 + Z_1)^2}$$

where X_n, Y_n, Z_n are the coordinates of a given point in a cartesian coordinate system in space. (1)

B) Calculation of the average stresses at the left ventricular equator.

Walker, Hawthorne and Sandler[13] have derived formulae for calculating the average meridional and hoop stress at the equator of the left ventricle.

The average meridional stress is the force per unit area acting at the midplane of the heart in the direction of apex-to-base length. This formula was derived by equating the meridional wall forces to the pressure loading assuming the ventricle to be a surface of revolution and expressed in terms of wall thickness.

$$\sigma_m = PR_i/2H\ (1 + H/2R_i) \quad (2)$$

σ_m = average meridional stress (Gm/cm²)
P = left ventricular pressure (Gm/cm²)
Ri = internal radius (cm)
H = wall thickness (cm)

The average hoop stress is the force per unit area in the circumferential direction. Walker et al. have derived an equation for the calculation of this stress at the equator, assuming bending moments and shear stresses may be neglected.

$$\sigma H = \frac{PR_i}{H}\left[\frac{1}{1 + (H/2R_i)\left(\frac{R_i}{B}\right)^2}\right]\left[1 - \tfrac{1}{2}\left(\frac{R_1}{B}\right)^2\right] \quad (3)$$

σH = average hoop stress (Gm/cm²)
P = left ventricular pressure (Gm/cm²)
R_i = internal radius (cm)
H = wall thickness (cm)
B = ½ length of the ventricle (cm)

C) Calculation of left ventricular internal volume. The internal volume of the left ventricle was calculated according to the method suggested by Hunter, Cothran and Hawthorne[14] using the prolate ellipsoid as a geometric model of the left ventricular chamber.

$$V = 4/3\pi\ (R_i)^3\ B \quad (4)$$

R_i = internal transverse radius (cm)
B = ratio of major to minor axis

RESULTS

The geometric changes accompanying contraction of the left ventricle were studied by the direct application of dimension transducers to the internal and external surfaces of the heart and by visualization of radiopaque markers using biplane cineradiography.

Figure 4 exhibits the changes in left ventricular circumference, wall thickness, pressure and the aortic pressure in a dog representative of those which were instrumented for such re-

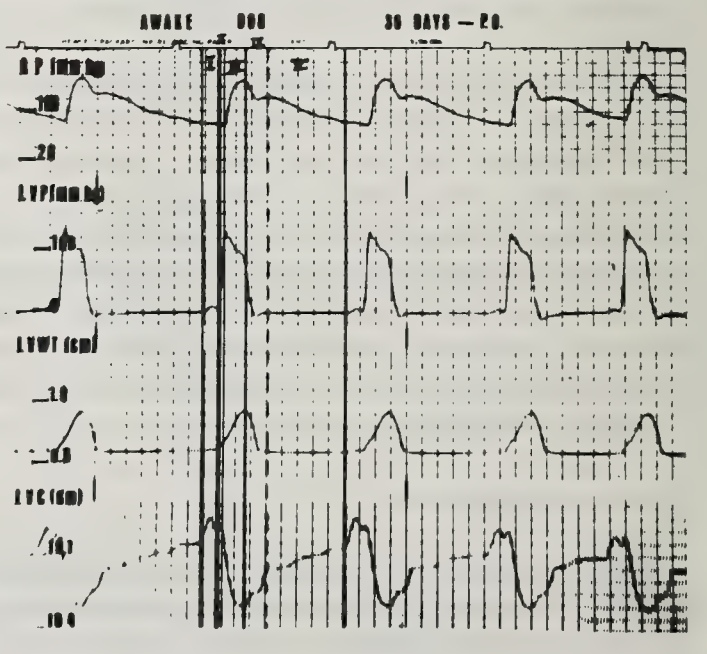


FIGURE 4.—A representative recording of aortic pressure (AP), left ventricular pressure (LVP), left ventricular wall thickness (LVWT) and left ventricular circumference (LVC).

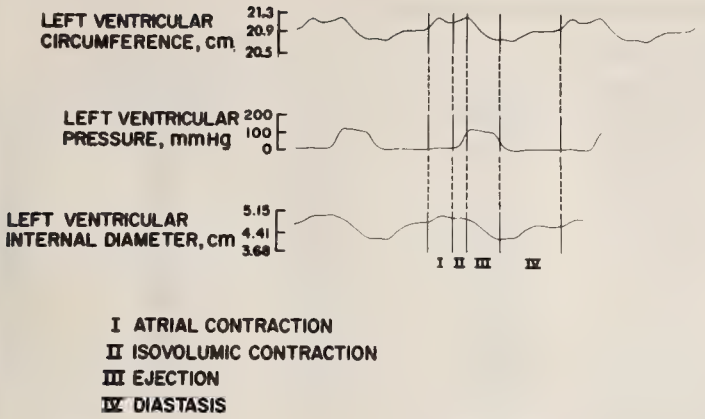


FIGURE 5.—A recording of transverse dimensions and ventricular pressure.

cordings. A recording obtained from one dog instrumented for measurements of left ventricular circumference, internal diameter and pressure is exhibited in Figure 5. Figure 6 is a recording of the cyclic variations in dimensions and pressures in a dog in which the external diameter and the wall thickness were measured by means of variable inductance gages. In each of the three animal preparations which were used in this study only two direct measurements of linear dimensions in the transverse plane were made. However, it was possible in each case, to derive other linear measurements in this plane from only two direct recordings. Figure 7 demonstrates four linear dimensions in the transverse plane of the left ventricle which may be obtained from the direct recording of an appropriate pair. In addition, by recording the left ventricular pressure it is possible to obtain an estimate of the force acting upon the wall. In the example shown (Figure 7), the left ventricular wall force was calculated as the product of pressure and internal cross sectional area.

The individual measurements of left ventricular transverse dimensions at selected times during a cardiac cycle are listed in Table I. The average end-diastolic external diameter in the group of dogs studied was 6.47 centimeters. A 3.55 per cent decrease in this dimension occurred as the left ventricle reached its end-systolic size. This change was associated with a 33 per cent increase in left ventricular wall thickness and a 21 per cent decrease in the internal diameter. From the linear measurements previously described several derived dimensions of the underlying rim of muscle were obtained at similar times during the cardiac cycle (Table II). The decline in the external diameter of the left ventricle during systole is associated with a rapid increase in wall thickness. This resulted in a marked increase (61.4%) in the estimated ratio of wall thickness to internal radius (thickness ratio). A 28 per cent increase in the cross sectional area of the left ventricular wall was found to occur and the internal circumference decreased by 22 per cent during systole (Table II).

The cyclic changes in left ventricular length were studied in five dogs by biplane cineradiography. Simultaneous recordings of the transverse dimensions and the intracavitary pressure of the left ventricle in these dogs were ob-

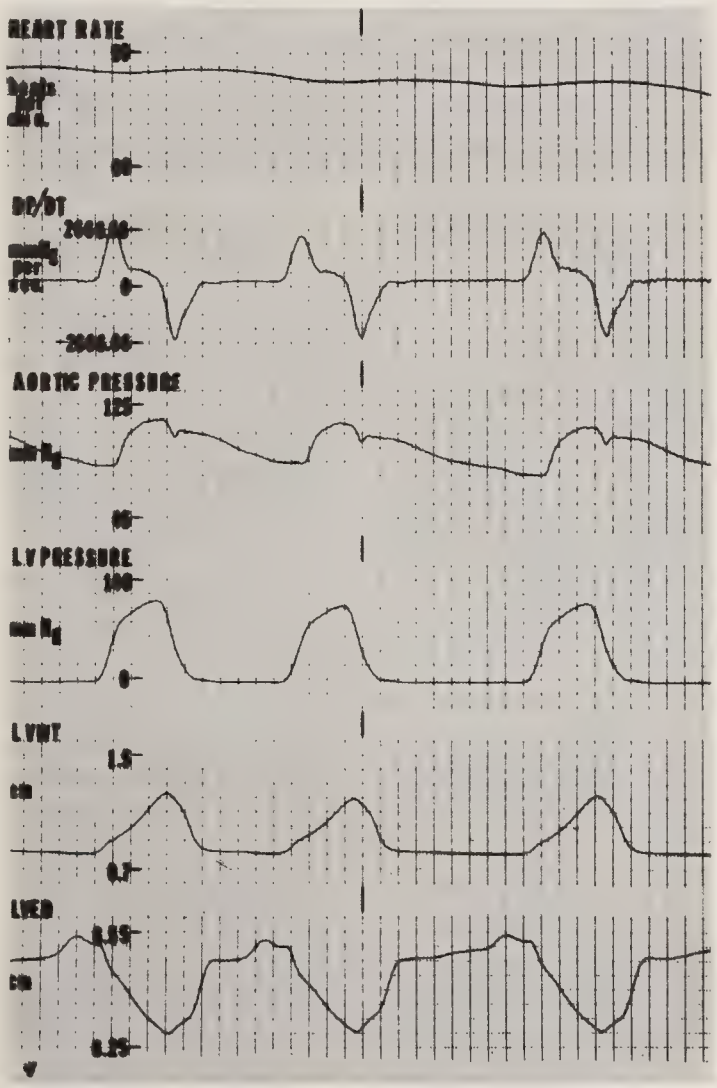


FIGURE 6.—A recording of left ventricular external diameter (LVED), wall thickness (LVWT), pressure and derived variables.

TABLE I.—*Left Ventricular Dimensions During the Cardiac Cycle*

Dog	External Diameter (cm)			Wall Thickness (cm)			Internal Diameter (cm)		
	(ED)	(EIVC)	(ES)	(ED)	(EIVC)	(ES)	(ED)	(EIVC)	(ES)
1	6.54	6.50	6.30	.879	.941	1.19	4.78	4.62	3.92
2	6.60	6.52	6.32	.921	.950	1.21	4.76	4.62	3.82
3	6.70	6.61	6.35	.970	1.00	1.43	4.76	4.61	3.49
4	6.57	6.50	6.25	.780	.880	1.28	5.00	4.74	3.69
5	6.23	6.24	6.00	.670	.750	1.06	4.89	4.74	3.88
6	6.50	6.45	6.20	.910	.940	1.19	4.67	4.56	3.82
7	6.44	6.42	6.20	.980	1.04	1.26	4.48	4.34	3.68
8	6.17	6.20	6.00	.970	1.15	1.40	4.23	3.90	3.20
9	6.22	6.27	6.09	1.02	1.12	1.48	4.18	4.03	3.13
10	6.75	6.77	6.55	.920	1.00	1.48	4.91	4.77	3.96
Av.	6.47	6.45	6.22	.900	.980	1.25	4.67	4.49	3.68

Key: (ED) end-diastole, (EIVC) end-isovolumic contraction, (ES) end-systole.

tained from implanted transducers. Figure 8 shows the temporal relationship of left ventricular pressure, length, external diameter and wall thickness from an animal of this group. The average length of the left ventricle at the beginning of systole in the group of dogs studied was 7.58 centimeters and the average end-systolic length was 7.12 centimeters (Table III). This decrease of 6.06 per cent in the length of the ventricle was considerably smaller than the associated reduction in the internal diameter (21%).

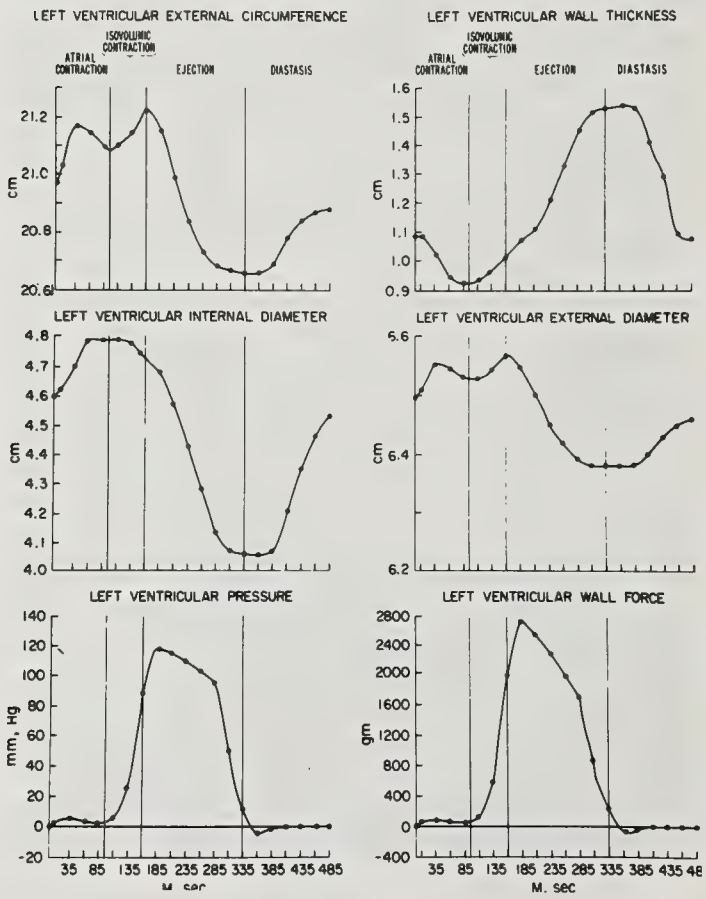


FIGURE 7.—A replot of left ventricular transverse dimensions, pressure and wall force.

By obtaining simultaneous measurements of the internal dimensions, wall thicknesses and pressures of the left ventricle we were able to calculate the average stresses generated by the myocardium throughout systole (Table IV). Plots of the average meridional stress and hoop stress and the corresponding left ventricular pressure are shown in Figure 9. The vertical arrows indicate the points of peak stress (P_s) and pressure (P_p). These forces are those occurring in an element of myocardium underlying the dimension gages at the equator of the left ventricle.

In addition to the foregoing possibility, simultaneous measurements of the internal dimensions of the left ventricle in two planes provided the required quantities necessary for examining the relationship between the major and minor axes of the chamber and for estimating the volume. Figure 10, panel 1 shows the curvilinear temporal relationship of the internal axis ratio during ventricular systole. Of the 22% average increase in the axis ratio occurring during systole (Table III), 14% of this amount occurred during ejection, and when the axis ratio was plotted against the internal transverse radius over this period, a linear relationship was obtained (Figure 10, panel 2). The data from the group of dogs, in which this relation was examined, were subjected to a linear regression routine and the line of best fit was found to be: $AR = -0.6Ri + 3$ (eq. 5) (Figure 11). The correlation coefficient for this line was 0.934 and the standard deviations of X and Y were $\pm$ 0.130 and $\pm$ 0.079, respectively.

TABLE II.—*Derived Dimensions During the Cardiac Cycle*

Dog	Thickness Ratio			Wall Area (cm^2)			Internal Circumference (cm)		
	(ED)	(EIVC)	(ES)	(ED)	(EIVC)	(ES)	(ED)	(EIVC)	(ES)
1	.370	.410	.610	15.64	16.42	19.10	15.00	14.50	12.30
2	.390	.420	.570	16.42	16.50	18.79	14.95	14.50	12.60
3	.410	.420	.820	17.46	17.58	22.15	14.95	14.50	11.00
4	.310	.370	.690	14.17	15.53	20.03	15.70	14.90	11.60
5	.270	.310	.550	10.89	12.93	16.44	15.35	14.90	12.20
6	.390	.410	.620	16.11	16.33	18.11	14.66	14.30	12.00
7	.440	.480	.680	16.80	17.57	19.55	14.07	13.60	11.50
8	.460	.590	.870	15.83	18.24	24.46	13.28	12.20	10.00
9	.490	.560	.950	16.64	18.17	21.47	13.12	12.60	9.80
10	.370	.420	.750	16.83	18.09	21.20	15.42	15.00	12.40
Av.	.390	.440	.710	15.68	17.64	20.13	14.60	14.10	11.50

Key: (ED) end-diastole, (EIVC) end-isovolumic contraction, (ES) end-systole.

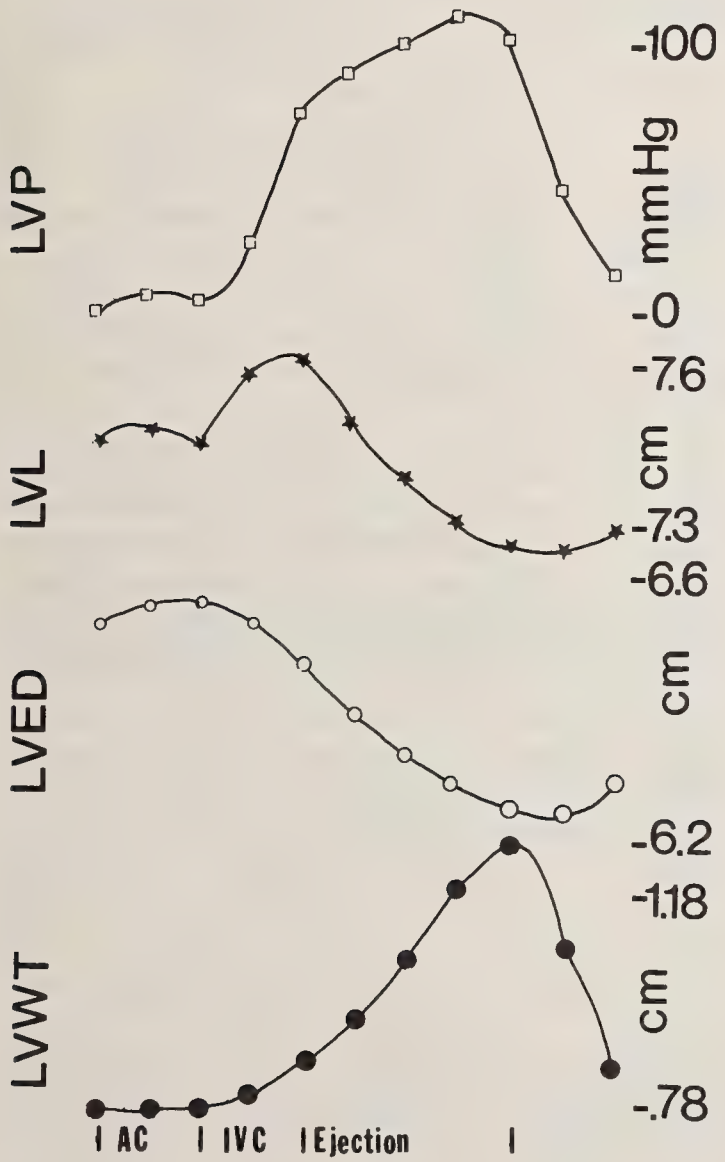


FIGURE 8.—Phasic variations of the left ventricular dimensions and pressure during the cardiac cycle. (LVL) left ventricular length, (LVED) left ventricular external diameter, (LVWT) left ventricular wall thickness.

In the animals in which the temporal change in axis ratio was measured, calculation of the left ventricular volume was possible. The actual numerical values for internal radius and axis ratio of the left ventricle were substituted in the equation for the volume of an ellipse of revolution (eq. 4), and approximations of left ventricular chamber volume were made at end-diastole and end-systole on five dogs (Figure 12).

The changes which we observed in left ventricular geometry indicated that ejection was the result of a decrease in the internal dimensions of the left ventricle in both the longitudinal and transverse planes. It should be emphasized that although the changes that occurred in length were significant (6%) (Table III), the decrease in the transverse plane was the most important dimensional change (21%) (Table I). Therefore, we examined the relationship between the changes in stroke volume and the internal radius of the left ventricle throughout the ejection period in a dog instrumented with an internal diameter gage and an aortic flow probe. We found, as did Bishop and Horowitz,[15] a linear relationship between these variables.

An example of the typical relationship between stroke volume and internal radius is illustrated in Figure 13. This experimental preparation also provided a convenient method to test the closeness of fit of our estimations of the changes in internal volume during ejection to actual values. To do this, we substituted the actual numerical value for the internal radius into the equation for the volume of a prolate ellips-

TABLE III.—*Changes in Left Ventricular Axes During the Cardiac Cycle*

Dog	Major Semi-Axis (cm)			Minor Semi-Axis (cm)			Axis Ratio		
	(ED)	(EIVC)	(ES)	(ED)	(EIVC)	(ES)	(ED)	(EIVC)	(ES)
1	3.58	3.81	3.50	2.39	2.31	1.96	1.50	1.65	1.79
2	3.66	3.70	3.53	2.38	2.31	1.95	1.54	1.61	1.85
3	3.57	3.73	3.53	2.38	2.30	1.80	1.50	1.62	1.96
4	3.82	3.89	3.67	2.50	2.37	1.84	1.53	1.64	1.94
5				2.44	2.37	1.94			
6	3.70	3.83	3.55	2.33	2.28	1.91	1.58	1.68	1.86
7				2.24	2.17	1.84			
8				2.11	1.95	1.60			
9				2.09	2.01	1.56			
10				2.45	2.38	1.98			
Av.	3.67	3.79	3.56	2.33	2.24	1.84	1.53	1.64	1.88

Key: (ED) end-diastole, (EIVC) end-isovolumic contraction, (ES) end-systole.

TABLE IV.—*Left Ventricular Pressures and Myocardial Stresses During the Cardiac Cycle*

Dog	L. V. Pressure (Gm/Cm^2)			Av. Meridional Stress (Gm/Cm^2)			Av. Hoop Stress (Gm/Cm^2)		
	(EIVC)	(APVP)	(ES)	(EIVC)	(APVP)	(ES)	(EIVC)	(APVP)	(ES)
1	120.0	155.0	53.5	122.9	136.6	33.8	224.8	254.1	68.2
2	128.5	170.0	51.3	133.0	127.6	31.3	242.5	245.6	63.8
3	119.7	179.5	59.8	116.3	148.1	26.8	211.9	265.0	59.8
4	106.3	158.8	63.9	121.3	99.3	35.1	217.7	209.7	76.5
5	123.8	172.7	62.6	168.6	215.8	40.1			
6	122.4	172.7	54.4	122.6	167.0	33.3	215.4	312.5	56.6
7	122.4	136.0	61.2	117.8	108.3	33.3			
8	140.1	150.0	68.1	64.3	71.4	27.0			
9	97.9	149.9	69.4	90.5	66.1	25.1			
10	129.2	163.2	61.2	119.0	141.1	29.8			
Av.	121.0	160.8	60.5	117.6	128.3	25.6	222.5	257.4	65.0

Key: (EIVC) end-isovolumic contraction, (APVP) at peak ventricular pressure, (ES) end-systole.

oid (eq. 4) and used a value for axis ratio which was calculated from the equation describing the linear relationship between axis ratio and internal radius (eq. 5). This was done at 20 msec intervals throughout ejection. In addition, similar calculations of volume were made where the end-diastolic axis ratio was determined and used as a constant in the moment-to-moment estimations. The results for a typical experiment are shown in Figure 14. The actual change in stroke volume (closed circles) was obtained by

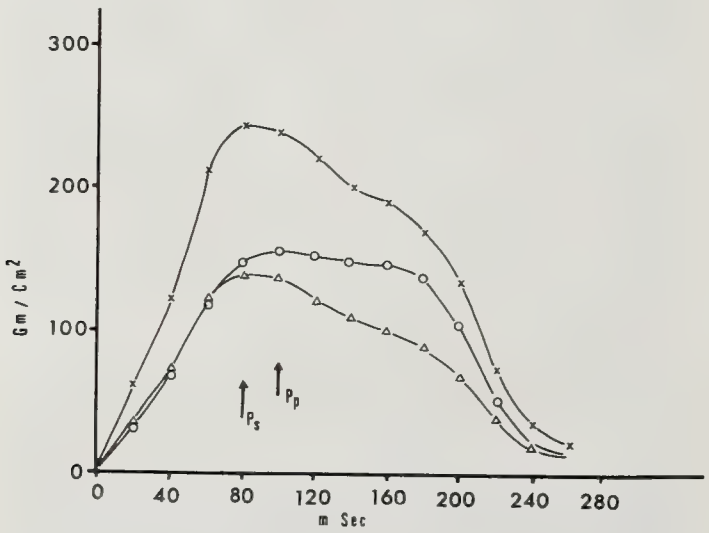


FIGURE 9.—The time course of left ventricular stresses and pressure. (X) hoop stress, (△) meridional stress, (○) left ventricular pressure.

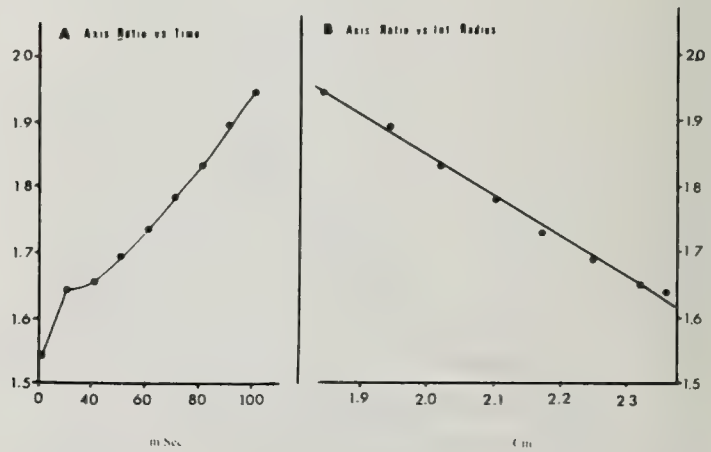


FIGURE 10.—(A) The time course of the left ventricular internal axis ration during ventricular systole. (B) The relationship between internal axis ratio and internal transverse radius during ejection.

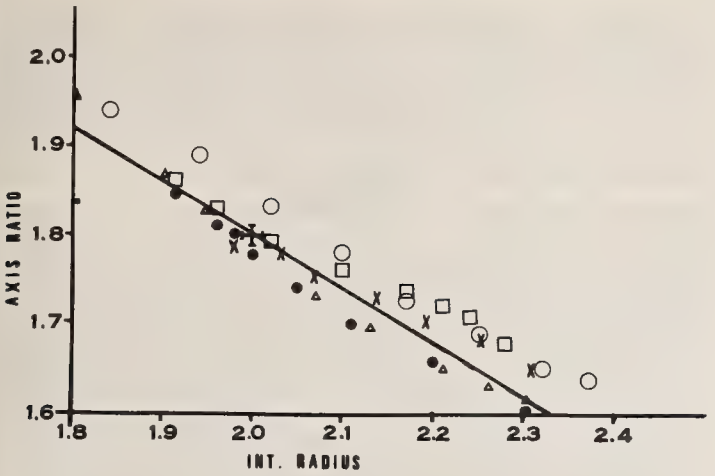


FIGURE 11.—The relationship between the internal axis ratio and internal radius of the left ventricle during ejection for the group of dogs studied.

electronic integration of the aortic flow curve. In each case, the calculated stroke volume closely approximated the actual value. The stroke volume obtained by assuming a constant axis ratio during ejection (triangles) overestimated the actual stroke volume (9.7%) while the value obtained by a point-to-point calculation of the axis ratio using equation 5, lies slightly below the true stroke volume (3.5%).

DISCUSSION

The dimensional changes accompanying contraction of the left ventricle were determined by the direct application of transducing devices to the heart and by the combined use of biplane cineradiographic and analog techniques. The phasic pattern of left ventricular dimensional changes demonstrated in the present study are consistent with the findings of other

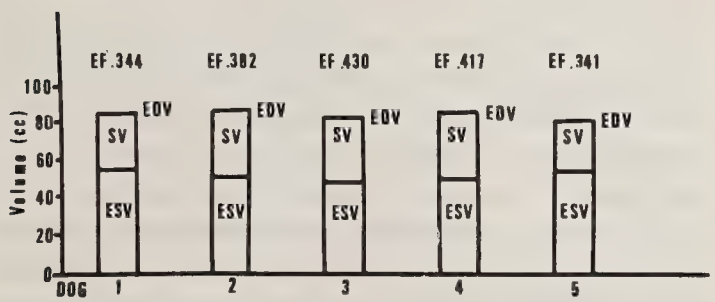


FIGURE 12.—Left ventricular volumes at end-diastole (EDV) and end-systole (ESV) for the group of dogs studied. (SV) stroke volume, (EF) ejected fraction.

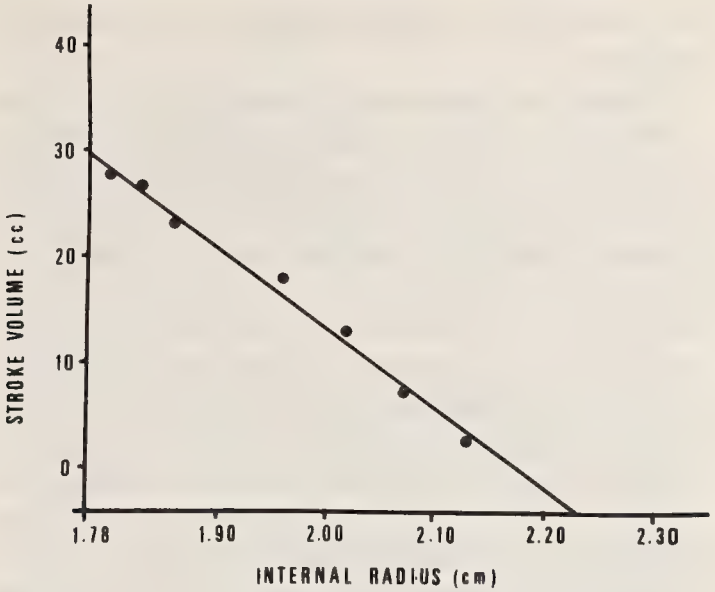


FIGURE 13.—A graph showing the linear relationship between stroke volume and the internal radius of the left ventricle.

investigators.[9,10,16-18] But, an attempt was made here to record simultaneously, the external, internal and transmural dimensional changes and to apply these data to estimates of myocardial stress and chamber volume. The equations used to calculate left ventricular stress and chamber volume are based upon the assumptions that the changes recorded in wall thickness were representative of the ring of muscle underlying the dimension gages and that

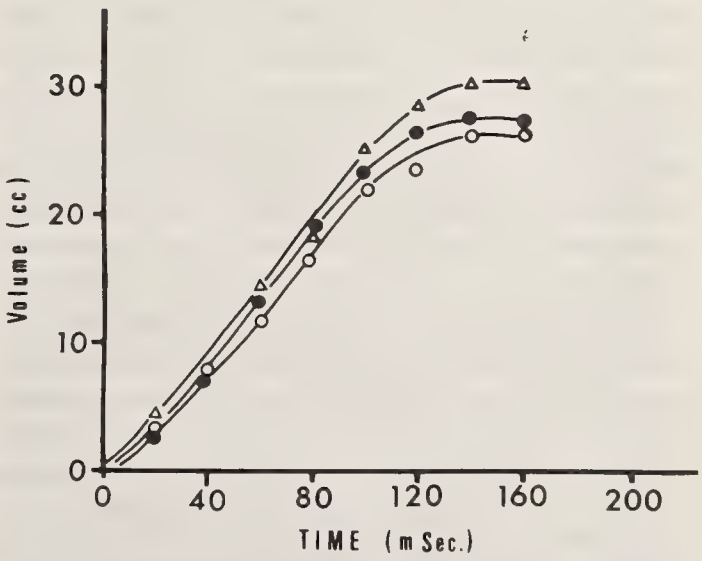


FIGURE 14.—Stroke volumes estimated using a constant axis ratio (△), a calculated axie ratio (○) and the curve obtained by electronic intergration of the aortic flow signal (●).

the ventricle in the plane of the minor axis was circular.

These data document the relative contributions of transverse and longitudinal dimensional changes to the geometric alterations occurring in the left ventricle during systole. The changes which we observed in left ventricular dimensions indicated that ejection resulted largely from a decrease in the internal diameter. The ratio of internal minor axis shortening to major axis shortening averaged 3.5:1 for the group of dogs studied. The ventricular lengths measured in these studies lie mainly along the outflow tract and exhibit agreement with the magnitude of aorta-to-apex length change which Hinds[9] observed. A consideration of the geometric alteration which must accompany these dimensional changes during ejection implies that the inner surface of the ventricle must decrease proportionately more than the external surface. This implied difference between external and internal dimensional changes is confirmed by the marked increase in wall thickness and cross sectional area seen at this time. Our measurements of thickness are in general agreement with those of other investigators.[10,19] The increase in wall thickness concommitant with a reduction in external diameter demonstrates that the major direction for wall thickening is toward the lumen.

The use of thin wall formulae for the measurement of tension or stress in various structures applies only if the ratio of wall thickness to internal radius is less than 0.1.[20] Our studies show that this ratio is considerably greater at end-diastole and increases markedly during ventricular systole. Therefore, we have used equations derived by Walker, Hawthorne and Sandler[13] which require thickness measurements in the calculation of meridional and hoop stress. The contour of the two stress curves obtained in this study are alike and bear a marked similarity to the left ventricular tension curves described by Sandler and Dodge[21] for humans and to the mural force curves described by Hefner[22] in anesthetized dogs. Of the two stresses which were calculated, the hoop stress was found to be the larger force. In fact, the maximum meridional stress is not significantly different from the magnitude of the peak left ventricular pressure when expressed in similar units of dimension (gm/cm^2). It is of interest that the average myocardial stresses are declining at a time when the left ventricular pressure is maximal and sustained. This occurs as left ventricular wall thickness approaches its maximum. The resultant increase in the cross-sectional area of the ventricular wall reduces the internal cross-sectional area to both lower the magnitude of the force acting at the endocardial surface and to provide a larger myocardial surface area across which this force is distributed.

The measurement of the internal diameter of the left ventricle provides a more useful way to get at estimates of internal volume than does the measurement of external dimensions. This is due to the significant changes in the thickness of the wall during the cardiac cycle and variations in this dimension at different sites. Bishop[15] has reported that a linear relationship exists between the moment-to-moment changes in the volume ejected from the left ventricle and the associated internal diameter. Likewise, we have observed this linear relationship in the present study. Bishop emphasized in his studies that this relationship is not dependent upon a constant geometrical shape of the left ventricle but rather a constant geometric factor. This is supported in part by our observation that the axis ratio increases during ejection while both the length and internal transverse diameter are decreasing. This finding indicates that the rate and extent of shortening of the minor axis exceeds that of the major axis. As a result, the ventricle assumes an increasingly prolate geometry during the time course of ejection.

The observation of a linear relationship between the axis ratio and the internal radius of the left ventricle during ejection made possible the calculation of the attendant internal volume from a knowledge of internal radius alone. This was done by substitution of the measured internal radius into the combination of equations 4 and 5 and by using the calculated end-diastolic axis ratio as a constant in equation 4. Estimations of the change in internal volume by the former method underestimates the actual stroke volume by only 3.5% while the latter method yields an overestimation of 9.7%. Both methods

appear to be meaningful approaches to calculation of the internal ventricular volume from end-diastole to end-systole in the canine.

SUMMARY

Experiments were designed and conducted to study the moment-to-moment changes in external and internal dimensions in conscious animals. The dimensions which were monitored included left ventricular circumference, external diameter, internal diameter and wall thickness in the mionor plane at the level of the equator of the heart. The chord length of the ventricle from the bifurcation of the coronary vessels to the apex was recorded simultaneously in five dogs by biplane cineradiography.

Analysis of the data obtained during normal cardiac cycles revealed that although the dimensions of the ventricular chamber decrease in both the longitudinal and transverse directions the reduction in internal diameter made the greater contribution to ejection. The increase in wall thickness during ventricular systole was found to be a major determinant of the decrease in internal cross-sectional area as well as a factor modifying the magnitude and time course of the myocardial stresses. A linear relationship between the internal diameter and stroke volume of the left ventricle was found; and a similar linear relationship was found when the internal radius was plotted against the time course of the changes in axis ratio throughout ejection. The equation describing this latter relationship was determined to be:

$$\text{Axis Ratio} = -0.6\ R_i + 3.$$

By use of this equation and the equation for volume of a prolate ellipsoid ($V = 4/3\pi\ R_i^3 AR$), it was possible to calculate the volume of the left ventricle at any point from end-diastole to end-systole using the measured internal transverse radius alone. The stroke volumes obtained by this procedure were found to underestimate the actual stroke volume by only 3.5%. Calculation of the stroke volume in this fashion but using the end-diastolic axis ratio as a constant, overestimated the actual stroke volume by only 9.7%.

REFERENCES

1. Laplace, P. S. Theorie de l'action capillarie. In: *Traite de Mecanique Celeste.* Supplement au livre x Paris 1805. Courcien.
2. Wood, R. H. A few applications of a physical theorem to membranes in the human body in a state of tension. J. Anat. et Physiol. 26:302, 1892.
3. Frank, O. On dynamics of cardiac muscle. Tr. by C. B. Chapman and E. Wasserman. Amer. Heart J. 58:282, 1959.
4. Rushmer, R. F. Shrinkage of the heart in anesthetized thoracotomized dogs. Cir. Res. 2:22, 1954.
5. Katz, L. N., Feinberg, H., and Shaffer, A. B. Hemodynamic aspects of congestive heart failure. Circulation 21:95, 1960.
6. Dodge, H. T., Sandler, H., Ballew, W., and Lord, J. D. The use of biplane angiocardiography for measurement of the ventricular volume in man. Amer. Heart J. 60:762, 1960.
7. Hawthorne, E. W. Dynamic geometry of the left ventricle. Amer. J. Cardiol. 18:566, 1966.
8. Gorlin, R., Rolett, E. L., Yurchak, P. M., and Elliott, W. C. Left ventricular volume in man measured by thermodilution. J. Clin. Invest. 43:1203, 1964.
9. Hinds, J. E., Hawthorne, E. W., Mullins, C. B., and Mitchell, J. M. Instantaneous changes in the left ventricular lengths occurring in dogs during the cardiac cycle. Federation Pro. 28:1351, 1969.
10. Cothran, L. N., Bowie, W. C., Hinds, J. E., and Hawthorne, E. W. Left ventricular wall thickness changes in unanesthetized horses. In: *Factors Influencing Myocardial Contractility.* Edited by: Tanz, R. D., Kavaler, F. and Roberts, J. New York: Academic Press, 1967, p. 163.
11. Horwitz, L. D., Bishop, V. S., Stone, H. L., and Stegall, H. F. Continuous measurement of internal left ventricular diameter. J. Appl. Physiol. 24:738, 1968.
12. Mitchell, J. H., and Mullins, C. B. Dimensional analysis of left ventricular function. In: *Factors Influencing Myocardial Contractility.* Edited by: Tanz, R. D., Kavaler, F. and Roberts, J. New York: Academic Press, 1967, p. 177.
13. Walker, M. L., Hawthorne, E. W., and Sandler, H. Methods for assessing performance for the intact hypertrophied heart. In: *Cardiac Hypertrophy.* New York: Academic Press, 1971, p. 387.
14. Hunter, F. K., Cothran, L. N., and Hawthorne, E. W. Early ventricular adjustments to a chronically increased afterload. In: *Cardiac Hypertrophy.* New York: Academic Press, 1971, p. 407.
15. Bishop, V. S., and Horowitz, L. D. Left ventricular transverse internal diameter: Value in studing left ventricular function. Amer. Heart J. 80:507, 1970.
16. Rushmer, R. F. Continuous measurement of left ventricular dimensions in intact unanesthetized dogs. Cir. Res. 2:14, 1954.

17. HAWTHORNE, E. W. Instantaneous dimensional changes of the left ventricle in dogs. Cir. Res. 9, 110, 1961.
18. WILSON, M. E. Left ventricular diameter, posture and exercise. Cir. Res. 11:90, 1962.
19. FEIGL, E. O., and FRY, D. L. Myocardial mural thickness during the cardiac cycle. Cir. Res. 14:541, 1964.
20. POPOV, E. P. *Mechanics of Materials.* Englewood Cliffs, N.J.: Prentice-Hall, 1952, p. 224.
21. SANDLER, H., and DODGE, H. T. Left ventricular tension and stress in man. Cir. Res. 13:91, 1963.
22. HEFNER, L., SHEFFIELD, T., COBBS, G., and KLIP, W. Ventricular force and pressure. Cir. Res. 11:654, 1962.

DISCUSSION

KENNETH L. GOULD, University of Washington, Seattle, Washington: When you put in your internal diameter measuring transducers, you have to lead the wires out through the ventricular wall. Now, depending on how one does this, a scar that forms around the wire may interfere with the normal thickening of the wall during systole. How do you circumvent that problem?

DR. COTHRAN: Yes. We were not able to circumvent it. We did use transducers where the cross-sectional area of the disc was considerably larger than the cross-sectional area of the wire. You do get a core in the center of connective tissue when making these measurements.

DR. GOULD: Do you think that where the wire went through the wall restricted the wall's ability to thicken in the internal diameter where you measured it?

DR. COTHRAN: In these studies, it may be restricted in measurements of internal diameter to some extent. However, in most of these animals, we monitored left ventricular wall thickness either directly where the gauges on the internal surface and external surface were coupled by means of an elastic band. We simply took precautions to make certain that there was no dimpling of the ventricle which would indicate that they were too tightly coupled.

DR. GOULD: The second question regards thick versus thin wall theories. In dogs, the results are very impressive. In humans, where the heart is larger, people have shown that if you calculate stress as you did, the error is not very large, 5 or 15% using the thick versus thin wall. In your experience, is this calculation appropriate for humans since the heart is larger, or is this difference primarily a function of size of the tissues that we're dealing with?

DR. COTHRAN: Well, first of all, the error which you get I think depends upon who made the calculation. I think Dr. Sandler has made calculations in humans and has indicated that you get something like a 30 to 60% error. Secondly, I think it would be dependent upon the ratio of wall thickness to internal dimensions. Having made no studies in humans, I don't know what these values are. But if it exceeds .1, then the thick wall theory should be used.

DR. GOULD: In humans, differences between thick and thin walls really aren't very large. It's also very interesting that in some 70% of our patients we get exactly the same stress curves as you do, and the relationship between myocardial stress and epicardial stress is very similar to yours and, in this sense, very comparable, but this again is using the thin wall theory. Would one of you comment comparing dogs and humans using the thick versus thin wall theory.

DR. COTHRAN: I think I'll defer to answer this.

DR. HAWTHORNE: Hal, do you want to take that one?

HAROLD SANDLER, NASA-Ames Research Center, Moffett Field, California: Your statement about measurement is indeed true, as long as you're dealing with absolutely one species, and as long as the people with coronary artery disease do not have left ventricular hypertrophy. Because calculations in the hypertrophied heart by the thin wall theory are going to be wrong, you will get the wrong answers. So it is difficult to say which is the right way. You must know the limits under which you're ordinarily operating. Some of your questions about the techniques that Dr. Cothran was using have been covered in papers published at a symposium of Federation pro-proceedings a year and a half ago. In that symposium two much more elegant techniques for studying dynamic geometry in cardiac systems were presented; one by Mitchell, where wet beads were inserted through the wall and radiographs were taken,

and the other by Carl, where screws were inserted into the endocardial surface. These methods, which supposedly "don't interfere with the wall with the wire coming through and problems with scarring," demonstrated findings identical to those presented here, which really substantiates that the methods used here are quite accurate.

A METHOD TO STUDY THE REGULATION OF CORONARY FLOW AND OF OTHER CARDIOVASCULAR FUNCTIONS IN THE UNANESTHETIZED PERFORMING DOG

Walter Ehrlich, Julij G. Tosheff, Paolo Caldini, David Krausman, Francine Schrijen and Joseph V. Brady*

A method is described for studying the regulation of coronary flow in the unanesthetized dog during the transition from rest to various standardized activities in a controlled environment. Flows in the circumflex branch of the left coronary artery and in the ascending aorta are recorded continuously. They are also integrated for each heart cycle and for each consecutive two-second interval. Systolic and diastolic blood pressure in the aorta, right atrial pressure, pleural pressure, and heart rate (beat-by-beat) are recorded simultaneously. The implantation of the sensors, the recording technique, the preprocessing for the computer analysis, and the statistical evaluation are described.

INTRODUCTION

Our laboratory tries to enhance today's understanding of the regulation of the systemic circulation, and especially of the coronary flow by studying the changes of circulatory functions during the transition from the steady state of rest to the steady states of various activities. We are interested in the direction, the extent, and the time sequence of the dynamic changes. The circulatory functions are, therefore, recorded continuously and evaluated in consecutive two-second intervals.

ANIMAL PREPARATION

Dogs are trained to stand still, to run, to eat, to drink or to perform other simple behavioral tasks in the experimental environment. When this training is completed, the animals are operated upon. All sensors to be implanted are gas sterilized. The operating table is covered with ultraviolet radiation.[1] The requirements of asepsis are strictly observed. The animals are narcotized (Barbithesal 650–800 mg. i.v.), intubated, respirated with a Harvard pump, and thoracotomized in the left fifth intercostal space (Figure 1). A flow transducer is implanted on the circumflex branch of the left coronary artery, and an occlusion cuff, as described by Gregg et al.,[2] is placed downstream from the transducer. Another flow transducer is attached to the ascending aorta. A 15 gauge polyvinyl chloride catheter is introduced into the right atrium through the left external jugular vein. An 18 gauge polyvinyl chloride catheter is inserted via the left carotid artery into the aorta with the tip of the catheter close to the aortic valve. A valve developed to measure pleural pressure changes[3] is fixed to the thoracic wall, and a 15 gauge polyvinyl catheter is slipped over the tube penetrating through the sixth intercostal space. The chest is closed. Air and fluid are evacuated through a rubber tube by inflating the lungs. The tube is then removed. The distal end of the catheters as well as the connectors of the flow transducers are brought out through the dog's back. When the dog is not in

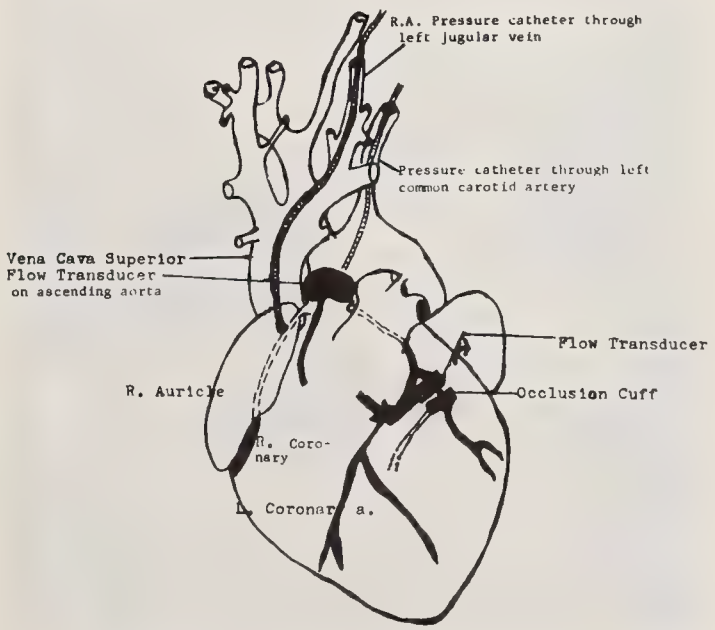


FIGURE 1.—Schema of preparation.

* Laboratory of Behavioral Cardiodynamics, Department of Psychiatry and Behavioral Sciences, School of Medicine; Department of Environmental Medicine, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205.

the experimental situation, the extruding part of the catheters and the connectors of the flow transducers are fixed to the dog's back by masking tape and covered with an elastic bandage around the trunk of the animal. A washable jacket protects the bandage. After the operation, the dog is prophylacticly treated with antibiotics for about one week. After one week, the stitches are removed.

From the third day after the operation, the training of the animal is resumed with the cables attached to the externalized connectors and with the pressure transducers fixed to the chest wall (Figure 2). The data used for research are recorded beginning two weeks after the surgery, when the flow transducers are well fixed by tissue reaction, the body temperature and weight as well as the hematocrit are at the preoperative level. From this time on we study the animal until one of the sensors stops functioning. This is usually due to a leak in the coating of one of the flow transducers.

THE RECORDINGS

During the experiments, the animal is in a sound-attenuated chamber. Outside of the chamber are a flowmeter (Biotronex 610), a recorder (Beckman R-dynograph), a speaker, a television set for the audiovisual monitoring of the animal, controls of the treadmill, a feeder, a fluid dispenser, an external pacemaker, (Medtronic 5.800), etc. The stabilization of the environment enhances the reproducibility of the results.



FIGURE 2.—Experimental animal.

The flow transducers (in Vivo metric) are calibrated prior to their implantation. The excursion levels of the integrators (Beckman couplers 9873B) are set to produce absolute values of flow in the monitored vessels. The zero flow level in the circumflex branch is obtained by occlusion of this vessel. The pressure channels and the cardiotachometer are calibrated before and after each experimental session. The 30 cm long pressure catheters attached to a Statham P37 pressure transducer have a natural frequency response in excess of 67 Hz with a damping ratio of 22% critical or better. The recording of the right atrial pressure and of the pleural pressure is filtered to exclude high frequency artefacts.

Figure 3 shows a paper strip recording of the monitored circulatory functions. The paper moves 25 mm. sec^{-1}. The instantaneous flow in the aorta is recorded on the first channel. The area limited by the curve of each beat and the base line indicates the amount of blood ejected into the aorta during that beat; that is, the stroke volume (less the coronary flow). The height of the deflection of the integrator on the second channel corresponds to the stroke volume. The height of the deflection of the integrator in the third channel corresponds to aortic flow in two seconds, or to $Qao.30^{-1}$ min. The instantaneous flow in the circumflex branch of the left coronary artery is recorded on the fourth channel. The area limited by the flow curve during one heart cycle and by the zero line is the amount of blood flowing in that coronary vessel during one heart cycle. The height of the deflection of the integrator in the fifth channel corresponds to the flow in that coronary vessel during one cycle. The height of the deflection of the integrator in the sixth channel corresponds to the amount of flow in that coronary vessel in two seconds. $Qcor.30^{-1}$ min. is believed to be indicative of flow changes in all coronary vessels; it is, however, actually measured in the circumflex

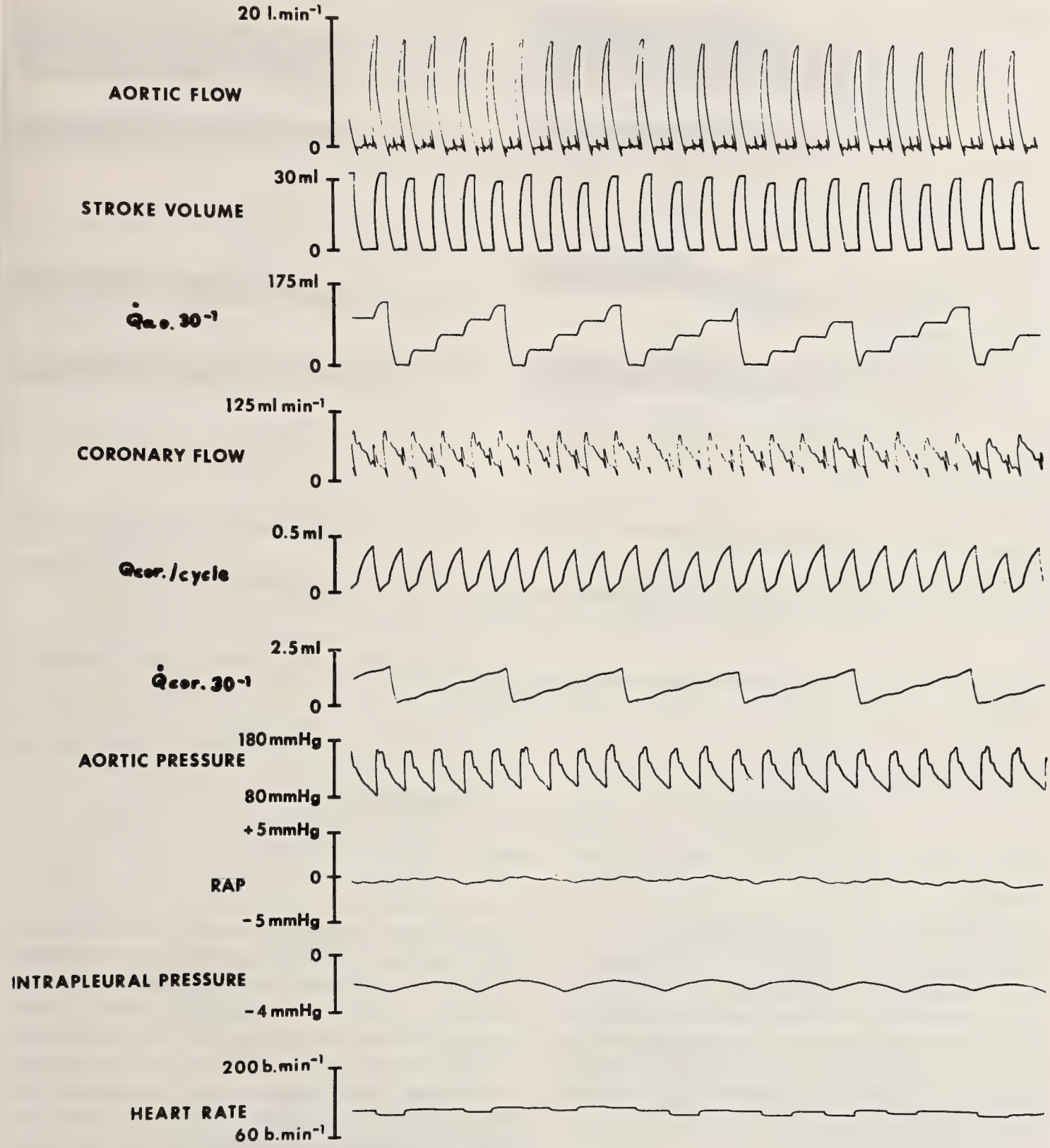


FIGURE 3.—Recording at 25 mm sec^{-1}.

branch of the left coronary artery only. The phasic blood pressure in the root of the aorta is recorded in channel seven. The eighth and ninth channels record the right atrial pressure and the pleural pressure changes, respectively. In the last channel, the heart rate is monitored by a cardiotachometer (Beckman 9875D) triggered by the onset of each ejection.

At the left side of Figure 4, the recording of the cardiovascular functions at rest and of their

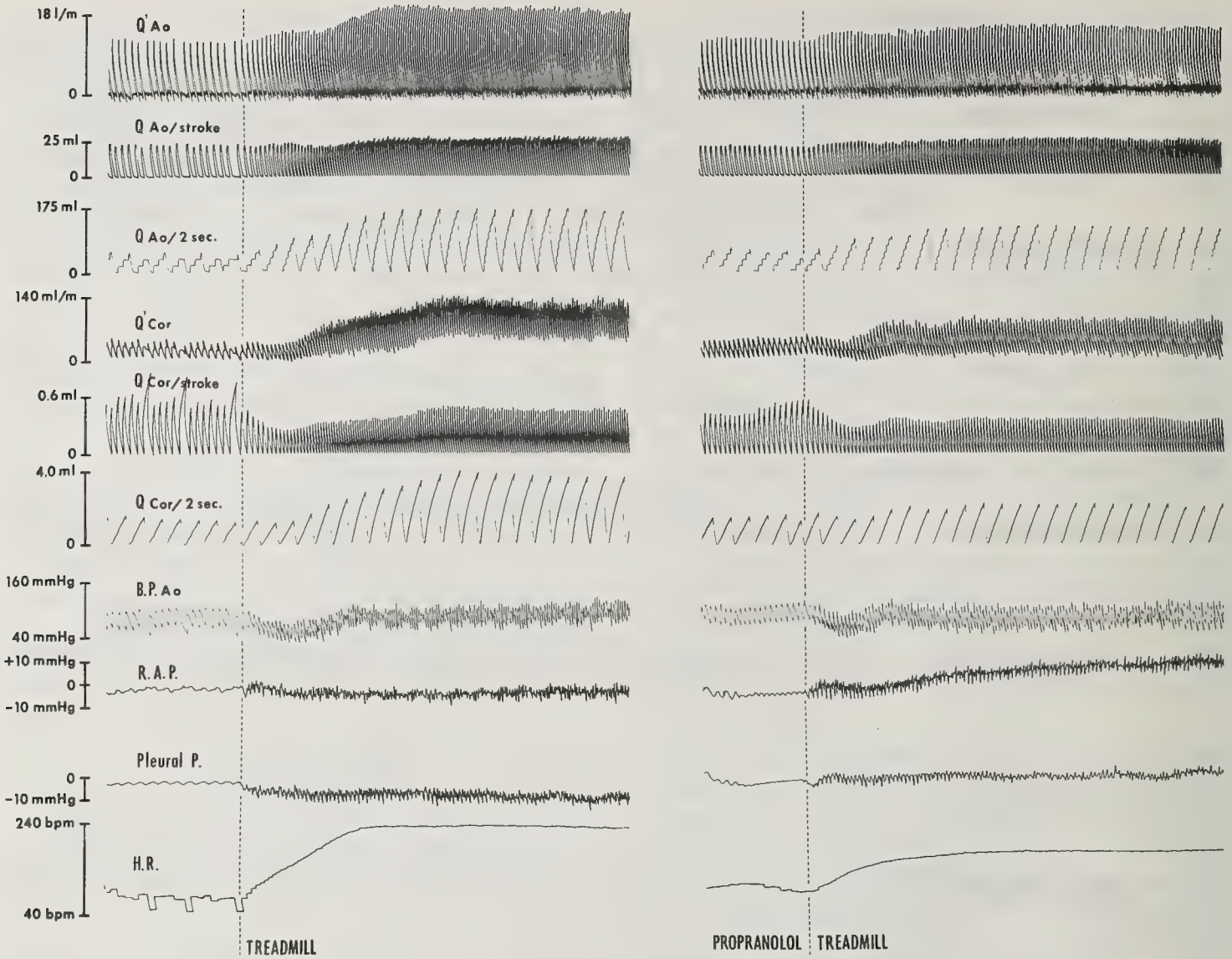


FIGURE 4.—Two recordings at 2.5 mm^{-1}.

evolving changes with the transition to exercise is reproduced at a paper speed of 2.5 mm sec^{-1}. Another recording of the cardiovascular changes during transition from rest to exercise is shown at the right side for comparison. This recording was obtained in the same experimental session after the β-blocker propranolol had been administered to the animal.

EVALUATIONS

We usually present individual recordings only as demonstration of experimental methods and/or illustration of results. The solution of research problems in our laboratory is, however, always based on mean values from all repeated experiments with each animal involved in a given project. For each animal we compute the mean rest values and the mean values for each of the fifteen successive two-second intervals of the studied activity. We do this for control conditions and again for the investigative conditions (for instance after propranolol administration). All of the results are tested for their statistical significance.

In order to be able to include the great amount of functional values into our evaluations, we feed the output of our implanted sensors not only to the Beckman R-dynograph, but also into a Sanborn Type 3907FM tape recorder. After completing the experiments, the monitored functions are played back from the tape re-

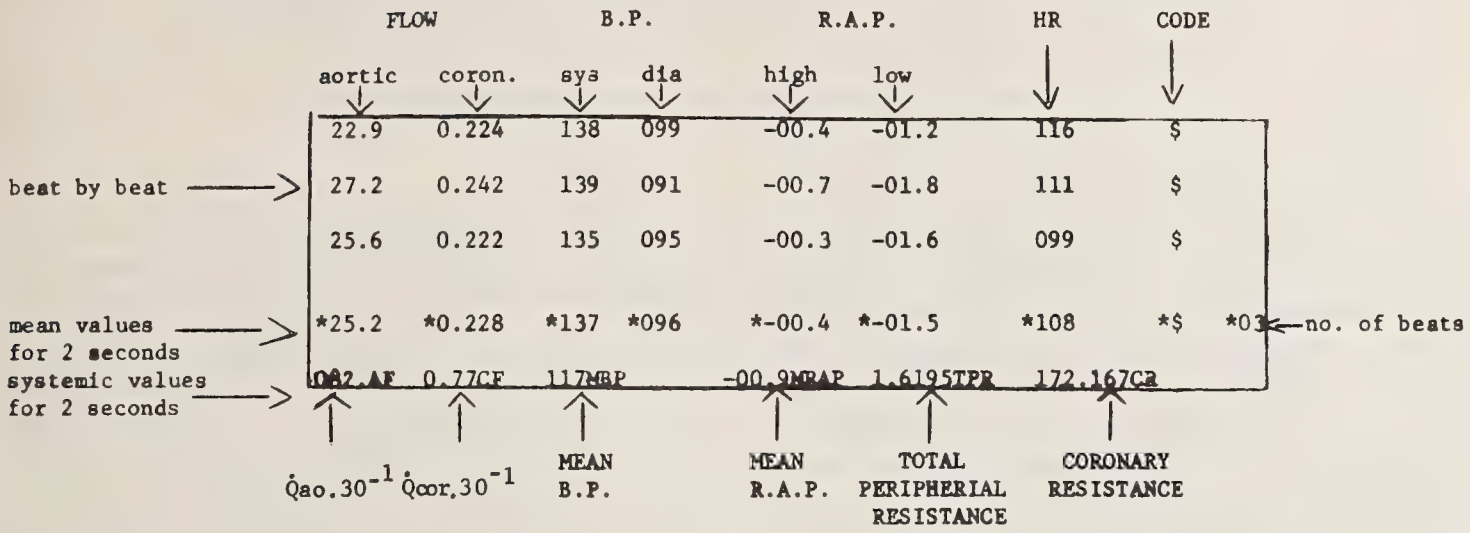


FIGURE 5.—Printout.

corder to the paper recorder. The data are preprocessed and fed through an interface into a Raytheon 709 processor with an A/D converter (Miniverter). The computing system gives the digital values for the flows, both peaks of the pressures, and the cardiotachometer value for each heart cycle. For the successive two-second intervals, it provides the values for mean flows, mean highest and lowest pressures, mean heart rate, and the actual number of beats. For the two second intervals the system prints also cardiac output ($Qao.30^{-1}$) coronary flow in the circumflex branch ($Qcor.30^{-1}$) mean blood pressure, mean right atrial pressure, total peripheral resistance and coronary resistance (Figure 5).

With this preparation, we have investigated the adaptation of the cardiovascular system and especially of the coronary flow to exercise and compared it with the cardiovascular adaptation to food intake[4] and to drinking.[5] We have studied the effect of β-blockade on the cardiovascular system in the unanesthetised organism.[6]

SUMMARY

With the progress of our work, we gain not only a better understanding of the regulation of the cardiovascular system but also more technical experience. Both again help us to improve our experimental model.

REFERENCES

1. RILEY, R. L., and PERMUTT, S. Room air disinfection by ultraviolet irradiation of upper air. Arch. Environ. Health 22:208, 1971.
2. GREGG, D. E., KHOURI, E. M., and RAYFORD, C. R. Systemic and coronary energetics in the resting unanesthetized dog. Circulation Res. 16:102, 1965.
3. TOSEV (TOSHEFF), J., DALEY, L. A., and HILTON, A. A method for recording intrapleural pressure in conscious, semi-restrained mammals. J. Appl. Phys. 27:902, 1969.
4. EHRLICH W., TOSHEFF, J., and BRADY, J. V. Difference between adaptation of coronary flow to treadmill exercise and to eating. Proc. Intern. Union Phys. Sci. 9:158, 1971 A.
5. EHRLICH W., TOSHEFF, J., CALDINI, P., and BRADY, J. V. Adaptation of cardiac output, coronary flow and other circulatory functions in dogs to drinking. Circulation 43 and 44:suppl. II, 161, 1971, B.
6. EHRLICH W., TOSHEFF, J., CALDINI, P., KRAUSMAN, D., SCHRIJEN, F., SANTOSO, S., and BRADY, J. V. Effect of β-adrenergic blocking on coronary flow and other circulatory functions of dogs at rest and during adaptation to exercise. Feder. Proc. 31:640, 1972.

RHEOLOGIC PROPERTIES OF BLOOD VESSELS

Joseph S. Janicki,* Dali J. Patel,** John T. Young** and Ramesh N. Vaishnav***

The blood vessels under consideration were the middle descending thoracic aorta (MDTA), the left circumflex coronary artery (LCCA) and the carotid artery (CA). Several assumptions concerning these vessels were necessary before the rheological properties could be determined. They are (1) homogeneity (uniform mechanical properties over a vessel length of 2 to 8 cm depending on the vessel studied); (2) cylindricity (no vessel taper and a circular cross section); (3) thin-walled (ratio of mid-wall radius to thickness is 10 or larger); (4) incompressibility (wall volume remains constant when subjected to large deformations); and (5) curvilinear orthotrophy (elastic symmetry about the planes perpendicular to the radial, circumferential and longitudinal directions). Under these assumptions, constitutive relations were developed whereby the static and dynamic, incremental, anisotropic moduli could be calculated from pressure, diameter, longitudinal force, length and wall volume measurements. In 14 dogs the static elastic properties of the LCCA and the CA were determined *in vitro* and in 14 other dogs the static elastic properties of the MDTA were studied *in vivo* and *in vitro*. Results indicate (1) both the LCCA and MDTA were more distensible than CA in the circumferential direction; (2) the LCCA was stiffer in the longitudinal direction than in the circumferential direction; the reverse was true of the CA; and (3) the elastic moduli of the MDTA increased with an increase in intravascular pressure. In 10 additional dogs the visco-elastic properties of the MDTA were also studied *in vivo* over the frequency range of 0 to 5 hertz. Results indicate (1) the longitudinal visco-elastic modulus to be the largest; (2) the storage moduli, in all directions, increased initially with frequency and then essentially remained constant; and (3) the loss moduli, in all directions, did not significantly change with frequency.

INTRODUCTION

The need to quantify the mechanical properties of blood vessels has long been recognized as a fundamental aspect of the understanding of cardiovascular hemodynamics. For example, knowledge of blood vessel rheology is necessary (1) in the design of vascular prosthesis and heart assist devices, (2) in the interpretation of hemodynamic data for diagnostic or therapeutic purposes, and (3) in the understanding of degenerative vascular diseases.

One of our primary objectives over the past ten years has been the development of an experimental technique which enabled us to measure the rheologic properties of blood vessels in the living dog. This technique required the adaptation of several basic engineering concepts to the biological system. In doing this, several simplifying assumptions had to be made in order to keep the mathematics tractable. Whenever possible, these assumptions were validated experimentally. Also, special instrumentation had to be designed and fabricated. To date, the canine middle descending thoracic aorta (MDTA) has been studied both dynamically and statically *in vivo* and *in vitro*, and in addition, the left circumflex coronary artery (LCCA) and the carotid artery (CA) have been studied statically *in vitro*. It is the purpose of this article to review this technique and the experimental results so obtained.

THEORY

This section condenses the extensive review given by Patel and Vaishnav.[1]

The blood vessel segment is taken to be a thin-walled circular cylindrical tube of constant thickness. At a given state of initial strain, the vessel wall is considered linearly elastic and curvilinearly orthotropic. The arterial tissue is assumed to be homogeneous and incompressible. The implication in assuming the vessel to be thin-walled is that the distribution of the circumferential stress across the thickness will be uniform. In engineering practice this assumption is made when the ratio of mid-wall

* Department of Medicine, University of Alabama, Birmingham, Alabama.

** Section on Experimental Atherosclerosis, National Heart and Lung Institute, N. I. H., Bethesda, Maryland.

*** Department of Civil and Mechanical Engineering, The Catholic University of America, Washington, D. C.

radius(R) to thickness(h) is around 10.[2] Typical blood vessel values of this ratio, at physiologic strains, are: 8 to 10 for MDTA and CA, and 6 to 8 for LCCA. The degree of error associated with this assumption cannot be easily evaluated.

Assuming the vessel to be a uniform cylinder implies that the diameter and thickness remain constant with length. The CA and LCCA segments approximated a cylinder satisfactorily. The descending aorta, however, tapers and the difference in end diameters of the 8 cm long segment was 1 to 2 mm. Thus, for a cylindrical blood vessel it is convenient to use the cylindrical coordinate system (r, θ, z) as a frame of reference. That is, the z coordinate corresponds to the center line of the lumen, the r coordinate to a vessel radius, and the θ coordinate to the vessel circumference.

The linearity assumption is based on the consideration that incremental strain perturbations around the physiologic state of strain produce linear responses provided these incremental strains remain within 4% of the initial strain. That is, within this 4% limit, the radius-pressure-longitudinal force relations and the length-pressure-longitudinal force relations are linear.

Patel and Fry[3] have shown that under physiologic loading, the shearing strains can be neglected. That is, the blood vessel may be treated as a curvilinearly orthotropic tube. This assumption reduces the number of independent constitutive parameters from 21 to 6, thus simplifying the analysis.

The homogeneity assumption implies that the mechanical properties over the vessel segment are uniform. Histologically, homogeneity is more justified for the circumferential and longitudinal directions than for the radial directions. Nevertheless, this assumption imposes a restriction on the results in that they represent average properties.

The final assumption of incompressibility has been justified by Carew, Vaishnav and Patel.[4] Incompressibility implies that when a vessel undergoes a change in strain, the volume of the vessel wall remains constant.

The external forces acting on the blood vessel segment are the intraluminal pressure (p) and an additional longitudinal force (F). With this kind of loading, and because the segment is homogeneous and a thin-walled cylinder, the only non-zero stresses are the normal stresses S_θ, S_z and S_r along the coordinate lines θ, z and r, respectively, and are given by

$$S_\theta = p\left(\frac{R}{h} - \tfrac{1}{2}\right) \tag{1a}$$

$$S_z = \frac{p}{2}\left(\frac{R}{h} - 1\right) + \frac{F}{2\pi Rh} \tag{1b}$$

$$S_r = \frac{-p}{2} \tag{1c}$$

Equations (1a) and (1b) result from equilibrium considerations, and equation (1c) equates the radial stress to the average of the pressure which is $-p$ on the inner surface and zero on the outer surface.

The state of strain corresponding to the above state of stress is described by the extension ratios λ_θ, λ_z and λ_r in the θ, z and r directions respectively. Because of orthotropy (3), under the given state of stress, there are no shearing strains. These ratios are given by

$$\lambda_\theta = \frac{R}{R_o} \tag{2a}$$

$$\lambda_z = \frac{L}{L_o} \tag{2b}$$

$$\lambda_r = \frac{h}{h_o} \tag{2c}$$

where R_o, L_o and h_o are the dimensions of mid-wall radius, segment length and vessel thickness, respectively, when $p = F = 0$ (unstressed state). L is the resulting length of the segment when the forces p and F are applied. Thus, the extension ratio is the ratio of a stressed dimension value to its unstressed value. Since the vessel wall is incompressible, the stretches must satisfy the condition

$$\lambda_\theta \lambda_z \lambda_r = 1 \tag{3}$$

Now suppose the segment is subjected to a small sinusoidally fluctuating pressure increment (Δp) and longitudinal force increment

(ΔF). Then the initial stresses will change by amounts ΔS_θ, ΔS_z and ΔS_r, and the initial stretches by amounts $\Delta\lambda_\theta$, $\Delta\lambda_z$ and $\Delta\lambda_r$. Again, because of orthotropy, no incremental shearing stretches will develop and the incremental stretches will completely define the incremental deformation. These incremental stretches must also satisfy the incompressible condition; therefore,

$$(\lambda_\theta + \Delta\lambda_\theta)(\lambda_z + \Delta\lambda_z)(\lambda_r + \Delta\lambda_r) = \lambda_\theta\lambda_z\lambda_r = 1 \qquad (4)$$

Let e_θ, e_z and e_r be the incremental strains defined by

$$e_\theta = \frac{\Delta\lambda_\theta}{\lambda_\theta}\ ;\ e_z = \frac{\Delta\lambda_z}{\lambda_z}\ ;\ e_r = \frac{\Delta\lambda_r}{\lambda_r} \qquad (5)$$

Or, equivalently, if ΔR, ΔL and Δh are the sinusoidally varying increments of R, L and h, respectively, then using equations (2) gives

$$e_\theta = \frac{\Delta R}{R}\ ;\ e_z = \frac{\Delta L}{L}\ ;\ e_r = \frac{\Delta h}{h} \qquad (6)$$

In terms of the incremental strains, equation (4) becomes

$$e_\theta + e_z + e_r = 0 \qquad (7)$$

when one assumes that the increments in stresses and stretches are sufficiently small so that they can be replaced by their corresponding differentials. Using equations (6) and (7) to express e_r in terms of e_θ and e_z, gives

$$e_r = \frac{\Delta h}{h} = -\left(\frac{\Delta R}{R} + \frac{\Delta L}{L}\right) \qquad (8)$$

Let P_θ, P_z and P_r denote the sinusoidally varying incremental stresses. By computing the differentials of the stresses in equation (1) and using equation (8) to eliminate Δh, the following relations for the incremental stresses are obtained:

$$P_\theta = \left(\frac{R}{h} - \tfrac{1}{2}\right)\Delta p + \frac{2p\Delta R}{h} + \frac{pR\Delta L}{Lh} \qquad (9a)$$

$$P_z = \left(\frac{R}{2h} - \tfrac{1}{2}\right)\Delta p + \frac{p\Delta R}{h} + \frac{\Delta F}{2\pi Rh} + \left(\frac{pR}{2Lh} + \frac{F}{2\pi RLh}\right)\Delta L \qquad (9b)$$

$$P_r = -\tfrac{1}{2}\Delta p \qquad (9c)$$

At this point in the development of the theory, the complex representation of the sinusoidally varying quantities should be discussed. Consider as an example the quantity Δp, represented by

$$\Delta p = |\Delta p| e^{j(\omega t + \phi)} = |\Delta p|\{\sin(\omega t + \phi) + j\cos(\omega t + \phi)\} \qquad (10)$$

Here $|\Delta p|$ is the maximum amplitude of the incremental pressure above the mean pressure (p) and ϕ is the phase angle which depends on when time (t) is taken to be zero. ω is the frequency in radians per second and $j = \sqrt{-1}$. We have arbitrarily chosen $t = 0$ to be that time when the phase angle of the complex quantity ΔR is zero. Thus, at $t = 0$, Δp, ΔR, ΔF and ΔL have the following complex representations:

$$\Delta p = |\Delta p| e^{j\phi} \qquad (11)$$

$$\Delta R = |\Delta R| \qquad (12)$$

$$\Delta F = |\Delta F| e^{j\alpha} \qquad (13)$$

$$\Delta L = |\Delta L| e^{j\psi} \qquad (14)$$

Since P_θ, P_z, P_r, e_θ, e_z and e_r are computed from Δp, ΔR, ΔF and ΔL, they too will be complex numbers. It is important to keep in mind that the static case (i.e., $\omega = 0$ and all phase angles become zero) is simply a special case of this dynamic formulation.

The complex incremental stresses and strains are related as

$$e_\theta = C_{\theta\theta}P_\theta - C_{\theta z}P_z - C_{\theta r}P_r \qquad (15)$$

$$e_z = -C_{z\theta}P_\theta + C_{zz}P_z - C_{zr}P_r \qquad (16)$$

$$e_r = -C_{r\theta}P_\theta - C_{rz}P_z + C_{rr}P_r \qquad (17)$$

where C_{ij} (i,j = r, θ, z) are complex incremental proportionality constants that relate the component of strain along the i direction to the corresponding stresses in the three j directions. The negative signs for C_{ij} ($i \neq j$) have been chosen for convenience. Inherent to these relations is the assumption of linearity within the range of the incremental strains. Also, note that this formulation is such that it allows the material to exhibit an incremental orthotropic response.

Assuming that C_{ij} in equations (15–17) obey the symmetry conditions

$$C_{zr} = C_{rz} \; ; \; C_{z\theta} = C_{\theta z} \; ; \; C_{r\theta} = C_{\theta r} \qquad (18)$$

and using equation (7), we have from equations (15–17)

$$C_{\theta\theta} - C_{z\theta} - C_{r\theta} = C_{zz} - C_{z\theta} - C_{zr} = C_{rr} - C_{r\theta} - C_{rz} = 0 \qquad (19)$$

which is used to express C_{ij} ($i \neq j$) in terms of C_{ij} ($i = j$). Thus,

$$C_{zr} = \tfrac{1}{2}(C_{zz} + C_{rr} - C_{\theta\theta}) \qquad (20)$$

$$C_{r\theta} = \tfrac{1}{2}(C_{rr} + C_{\theta\theta} - C_{zz}) \qquad (21)$$

$$C_{\theta z} = \tfrac{1}{2}(C_{\theta\theta} + C_{zz} - C_{rr}) \qquad (22)$$

Finally, using equations (18) and (20–22), we can write equations (15–17) in terms of $C_{\theta\theta}$, C_{zz} and C_{rr}. That is,

$$e_\theta = (P_\theta - \tfrac{1}{2}P_z - \tfrac{1}{2}P_r)C_{\theta\theta} + \tfrac{1}{2}(P_r - P_z)C_{zz} + \tfrac{1}{2}(P_z - P_r)C_{rr} \qquad (23)$$

$$e_z = \tfrac{1}{2}(P_r - P_\theta)C_{\theta\theta} + (P_z - \tfrac{1}{2}P_\theta - \tfrac{1}{2}P_r)C_{zz} + \tfrac{1}{2}(P_\theta - P_r)C_{rr} \qquad (24)$$

$$e_r = \tfrac{1}{2}(P_z - P_\theta)C_{\theta\theta} + \tfrac{1}{2}(P_\theta - P_z)C_{zz} + (P_r - \tfrac{1}{2}P_\theta - \tfrac{1}{2}P_z)C_{rr} \qquad (25)$$

Only two of these three complex equations are independent. Thus, we have three complex unknowns and two complex equations. In order to solve for all unknowns another independent state of incremental strain, around the same initial strain, is required to provide two more independent complex equations. These two states of incremental strains should yield significantly different values of the strain ratios $\frac{e_\theta}{e_z}$ and $\frac{e_\theta}{e_r}$. This requirement of two strain states formed the basis of our experimental procedure. It should be noted that we now have four complex equations in three complex unknowns of which any three equations could be used. However, it is also possible using a method of least squares to use all four equations. We decided to use the latter method. When presenting results, complex incremental elastic moduli, similar to the more familiar Young's modulus, are given instead of C_{ij}. These moduli are calculated from the complex incremental proportionality constants using the following general relations:

$$E_i' = \frac{C_{ii}'}{(C_{ii}')^2 + (C_{ii}'')^2} \; ; \qquad E_i'' = \frac{C_{ii}''}{(C_{ii}')^2 + (C_{ii}'')^2} \qquad (26)$$

where the prime and double prime quantities denote the real and imaginary parts, respectively, of the complex quantities. E′ is referred to as the storage modulus and E″, the loss modulus.

EXPERIMENTAL PROCEDURE

At attempt will be made to keep the procedural description general. If specific detail is required, the reader is referred to references 5 and 6. The vessel to be studied was sufficiently exposed so that a reasonably uniform segment of length, L, could be measured and marked. During this time, the aortic pressure was continuously monitored. Two cylindrical plugs, chosen to snugly fit the vessel lumen at each end of the segment, were inserted and secured. Figure 1 depicts the experimental setup used to study the LCCA and CA. Both of these segments were removed from the dog and stripped of their surrounding tissues up to the adventitia. A hollow metal rod (N) was

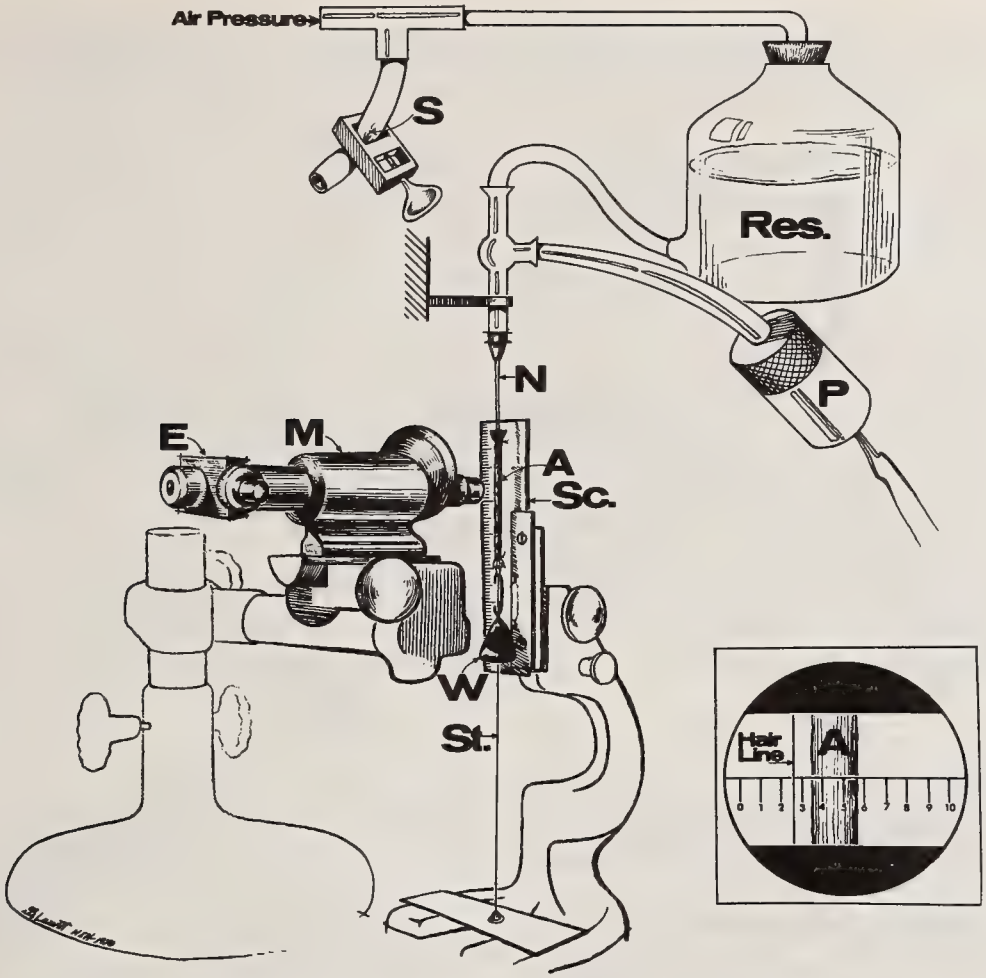


FIGURE 1.—Typical experimental setup to study the left circumflex coronary artery and the carotid artery. A = artery; Sc = centimeter scale; W = weight; St = string; M = microscope; E = Filar micrometer eyepiece; N = hollow metal rod; P = pressure transducer; Res = reservoir; S = screw clamp. Insert represents the view of the artery through the microscope. From Patel, D. J., and Janicki, J. S. Static elastic properties of the left coronary circumflex artery and the common carotid artery in dogs. Circ. Res. 27:149, 1970.

screwed into a threaded hole through the center of the proximal plug. The other end of this rod was connected to a reservoir (RES) containing a 6% dextran solution. The reservoir was made airtight and connected to an adjustable source of air pressure. Pressure in the segment (A), applied via this arrangement, was measured using a P–23 Db Statham transducer (P). A solid metal plug containing a hook was screwed into the distal plug so that weights (W) could be hung from it. A centimeter scale (SC) divided in half millimeter increments was mounted on a stand behind the segment. The length (i.e., the distance between the ties coupling the blood vessel to the plugs) and mid-segment external diameter of the blood vessel segment were measured using the microscope system (M), which was equipped with a Bausch and Lomb Filar micrometer (12.5x) eyepiece (E). The microscope system was mounted on a stand which was adjustable in both the horizontal and vertical directions. The eyepiece had a moveable hairline controlled by a vernier knob, which could be used to average the vessel diameter over a length of approximately 4 mm.

Figure 2 shows the experimental setup that was used to study statically the MDTA, both *in vivo* and *in vitro*. The setup for the dynamic studies was essentially the same except for the

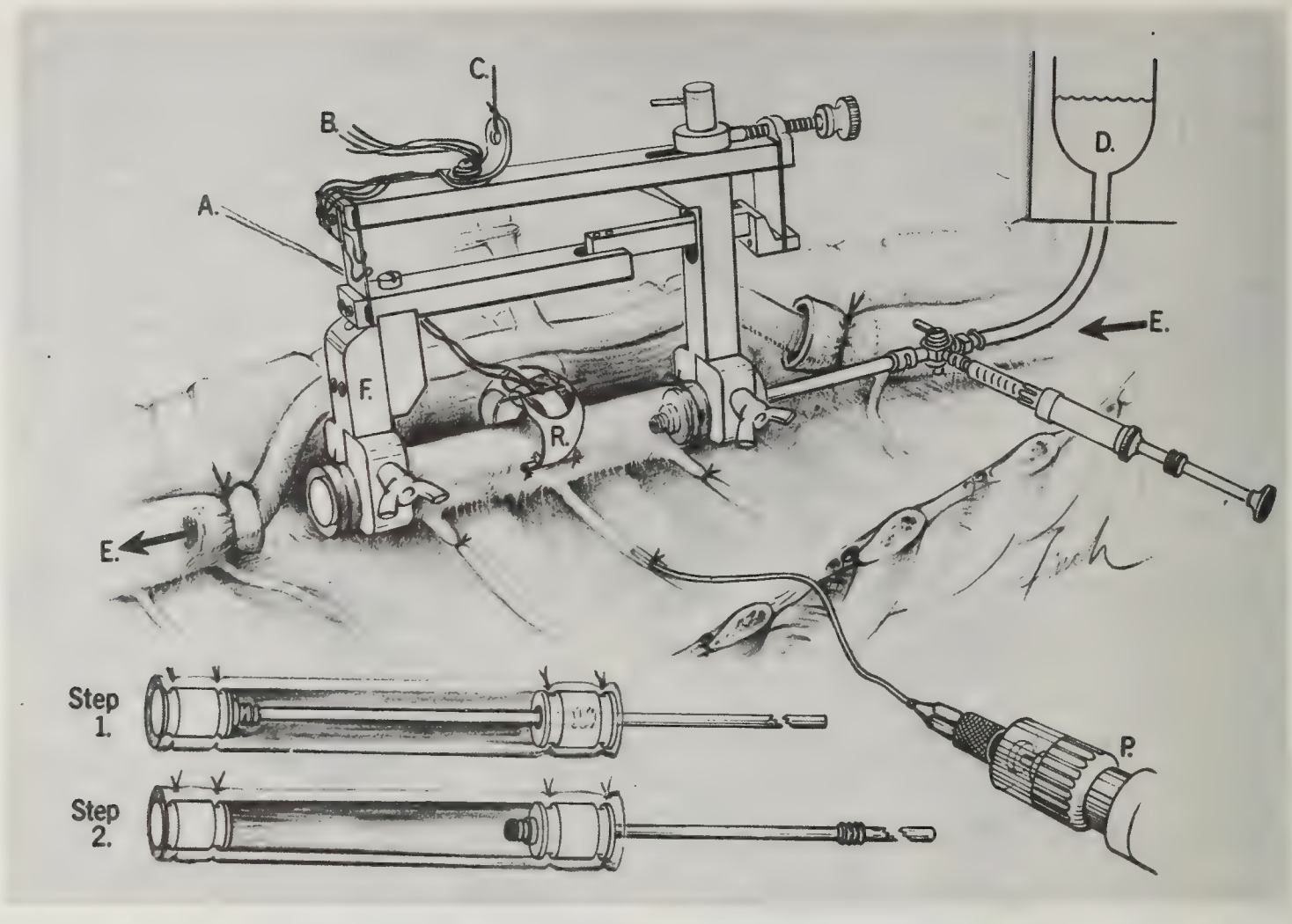


FIGURE 2.—Typical experimental setup to study middle descending thoracic aorta. A and B = lead wires from electrical calipers and the force gauge, respectively; C = supporting bracket; D = reservoir; E = descending thoracic aorta with bypass; F = variable length-force gauge device; P = pressure transducer; R = radial displacement sensing device. From Patel, D. J., Janicki, J. S., and Carew, T. E. Static anisotropic elastic properties of the aorta in living dogs. Circ. Res. 25:765, 1969.

following: The variable length-force gauge device (F) was modified so that the proximal leg could be moved sinusoidally; and (2) a sine wave fluid displacement pump was connected to the distal end of the segment via a rigid tube so that the segment volume could be sinusoidally varied. As seen, the segment was isolated and bypassed from the main systemic circulation (E position in Fig. 2). The proximal end of the segment was connected to a reservoir of oxygenated blood (D), the height of which maintained constant pressures in the vessel segment. Lateral pressure was measured at the mid-portion of the segment either through a cannulated intercostal artery (static studies) or through a thin needle placed coincident with the longitudinal axis (dynamic studies). The remaining intercostal arteries were ligated. A displacement sensing device (R) for continuous measurement of radius was sutured to the mid-portion of the pressurized segment. Finally, the variable length-force gauge device was attached to the plugs.

Prior to recording data, hysteresis effects were minimized by inflating and deflating the vessel segment for two cycles over the pressure range of 20 to 200 cm H_2O. Following this, the vessel was stressed to physiologic values by (1) adjusting the pressure to that value at which the vessel was measured and marked, and (2) adding an additional longitudinal force to restore the vessel length to L. In accordance with

the theoretical requirement of two strain states, the basic experiment consisted of two parts: (1) Variable length-constant volume (i.e., predominantly a longitudinal strain state); and (2) Constant length-variable volume (i.e., predominantly a circumferential strain state). Experimentally, Part 1 was accomplished by varying the length an amount ΔL, either statically or dynamically, around its initial value L and recording the resulting change in pressure (Δp), external radius (ΔRe) and force (ΔF), while keeping the volume constant; and Part 2 consisted of varying the volume, statically or dynamically, and again recording Δp, ΔRe and ΔF, while keeping the length constant. When doing a static experiment, stress-relaxation, following a perturbation, was allowed to occur by waiting one minute prior to data collection. In the dynamic experiments, data were recorded at 6 frequencies—0.5, 1, 2, 3, 4 and 5 hertz.

At the end of the experiment, the blood vessel segment was slit open longitudinally and the unstressed length (L_O) and circumference were measured. The value of the unstressed radius (R_O) was obtained by dividing the value of unstressed circumference by 2π. The wall volume, V, was then obtained by either weighing the specimen (LCCA and CA) and dividing by tissue density (1.06 g/cm^3) or determining the specimen's (MDTA) loss of weight when suspended in distilled water. Since the material is incompressible, V was a constant and the value of thickness, h, was calculated for different values of R_e and L from

$$h = R_e - \sqrt{\left(R_e^2 - \frac{V}{\pi L}\right)} \qquad (27)$$

The quantity $\frac{h}{2}$ was then subtracted from the measured external radius to obtain the corresponding mid-wall radius, R.

The dynamic data, which were recorded on analog tape, were digitized at a rate of 500 samples/second. Then, for each frequency, these data were subjected to Fourier analysis, whereby the phase angles ϕ, α and ψ were obtained. Thus, it is seen that all of the data required to calculate either the static or dynamic incremental strains and stresses as given by equations (6), (8) and (9) have been obtained. These quantities were calculated for both strain states and substituted into equations (23) and (24). The resulting system of four equations in three unknowns was then solved using a method of least squares. Finally, the solution, C_{ij} (i = j = r, θ, z), was converted into incremental moduli using equations (26).

RESULTS AND DISCUSSION

The static *in vitro* results for the MDTA, LCCA and CA are summarized in Table I. It is important to point out that the elastic moduli given for LCCA and CA are considered to be approximate for the following reasons: (1) the LCCA ratio $\frac{R}{h}$ is small and could introduce errors in the calculation of wall stress based on thin-wall theory; (2) the computations are sensitive to error in the measurement of radius and to errors due to the inability to keep length exactly constant during the variable volume-constant length part of the experiment; and (3) the measurement of wall volume of these segments is very difficult and consequently leads to errors in the calculation of wall thickness. These considerations would account for the large scatter in the elastic moduli values.

In spite of these reservations, it is reasonable to conclude the following: (1) both LCCA and CA demonstrate anisotropic behavior in the physiologic range; (2) both the MDTA and LCCA are more distensible than the CA in the circumferential direction; (3) the LCCA is

TABLE I.—*Summary of Static, Vitro Data*

	MDTA	LCCA	CA
No. dogs	6	5	5
P_m (dyne/cm^2) $\times 10^{-3}$	149 ± 2	164 ± 2	176 ± 9
L (cm)	7.9 ± .1	1.97 ± .09	3.63 ± .11
R_{mean} (cm)	0.677 ± 0.024	0.145 ± .01	0.232 ± .004
R/h	9.5 ± 1.0	6.71 ± .53	9.50 ± 1.28
λ_z	1.50 ± 0.07	1.53 ± .05	1.63 ± .04
λ_θ	1.48 ± 0.06	1.58 ± .09	1.50 ± .07
E_θ (dyne/cm$_2$) $\times 10^{-3}$	7600 ± 900	7700 ± 2200	14800 ± 3100
E_z (dyne/cm^2) $\times 10^{-3}$	6900 ± 900	38000 ± 11000	8700 ± 2000

Average values ± SE are shown. MDTA = middle descending thoracic aorta; LCCA = left coronary circumflex artery; CA = right common carotid artery; P_m = mean pressure; L = length; R_{mean} = mean value of midwall radius, R; h = thickness. λ_z and λ_θ are the extension ratios in longitudinal and circumferential directions. E_θ and E_z are the incremental elastic moduli in circumferential and longitudinal directions.

stiffer in the longitudinal direction than in the circumferential direction; and (4) the CA is stiffer in the circumferential direction than in the longitudinal direction.

The values of elastic moduli for the MDTA were found to decrease when the vessel segment was removed from the animal and restudied *in vitro*. The longitudinal modulus decreased by 32% ($p < .01$), while E_r decreased by 20% ($p < .05$) and E_θ by 9% ($0.5 < p < 0.1$). Thus, the *in vivo* relation between E_θ and E_z is just the opposite of that indicated in Table I. The 32% decrease in the value of E_z can be explained by the removal of the *in vivo* longitudinal tethering of the vessel segment. Using data from a previous study,[7] the contribution of vascular tethering to the *in vivo* longitudinal modulus is calculated to be 40%.

Figure 3 shows the relation of the *in vivo*, static moduli and the extension ratios λ_θ and λ_z. That is, the moduli are plotted against the mean values of three groups of λ_θ (upper panels) and against the mean values of three groups of λ_z (lower panels). It is seen that (1) the average

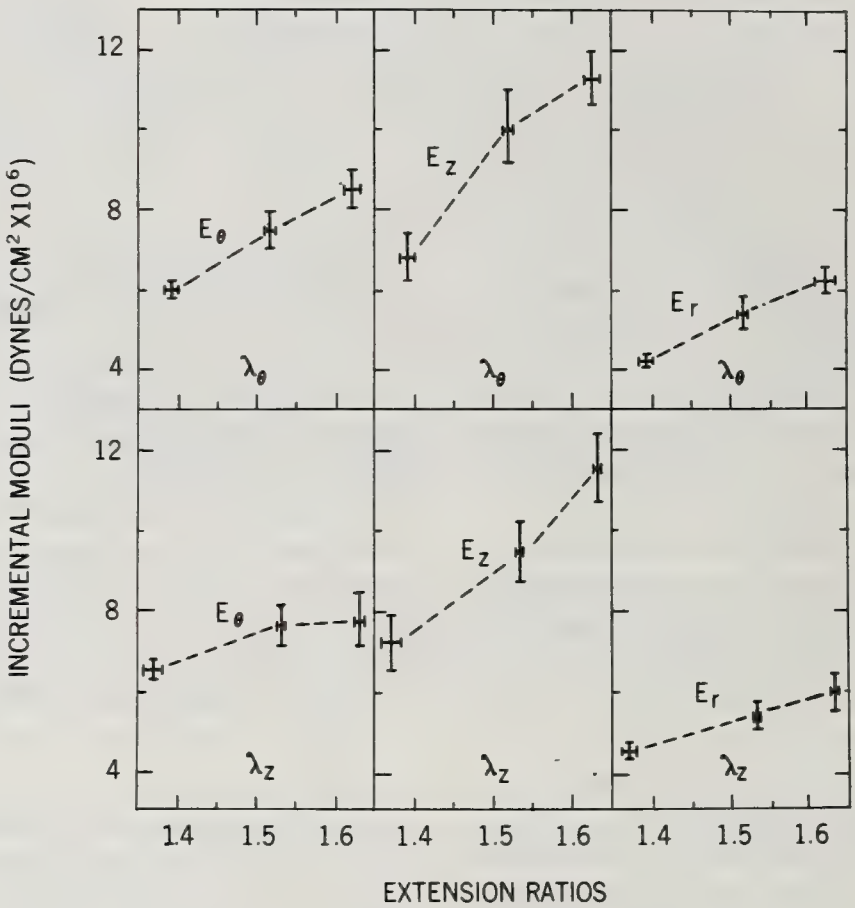


FIGURE 3.—Incremental elastic moduli and extension ratios. E_θ = circumferential modulus; E_z = longitudinal modulus; E_r = radial modulus; λ_θ = circumferential extension ratio; λ_z = longitudinal extension ratio; horizontal bars indicate the standard error of the mean for each of the groups. In the upper panels, the moduli are plotted vs. the mean λ_θ for each of three groups; the corresponding values of λ_z (from left to right) are 1.45 ± .04 SE, 1.56 ± .02, and 1.51 ± .02. In the lower panels, the moduli are plotted vs λ_z; the corresponding values of λ_θ for each of these groups (left to right) are 1.46 ± .02, 1.58 ± .02 and 1.48 ± .02. Adapted from Patel, D. J., Janicki, J. S., and Carew, T. E. Static anisotropic elastic properties of the aorta in living dogs. Circ. Res. 25:765, 1969.

value of the elastic moduli in the third group were always higher ($p < 0.01$) than in the first group, and (2) in general $E_z > E_\theta > E_r$ for *in vivo* physiologic strain states. This indicates that the MDTA exhibits an anisotropic behavior *in vivo*.

Typical results from a dynamic, *in vivo* study are given in Table II. The vessel was evaluated around a mean pressure of 140 cm H_2O and around an initial state of strain given by $\lambda_\theta = 1.41$ and $\lambda_z = 1.62$. The following points are noteworthy: (1) the longitudinal viscoelastic modulus is the largest; (2) the values of E_θ, E_z and E_r increase initially with frequently (0 to 2 hertz) and then essentially remain constant; and (3) E'_θ, E'_z and E'_r are much greater than E''_θ, E''_z and E''_r.

It should be reemphasized that the above results are valid only around the given physiological states of strain, and any other state of strain will require a different characterization of the vessel properties. Thus, when comparing data from various sources, one should always be certain of where on the large, nonlinear, stress-strain curve the data were obtained. A nonlinear theory for large elastic deformation, which in effect removes this restriction, has recently been developed.[8] This theory provides (1) a constitutive relation relating the stresses in an artery directly with the state of initial strain and (2) the values of the incremental moduli around any state of large strain. However, under its present stage of development, the viscoelastic (dynamic) case has not been incorporated. Applying this theory to the static *in vivo* MDTA data resulted in agreement within 10% for E_θ and E_z and 16% for E_r. Furthermore, in a set of *in vitro* experiments in which measurements were made over a wider range of strains, the agreement was within 7% for all three moduli.

TABLE II.—*Typical, Viscoelastic Results from the Canine Middle Descending Thoracic Aorta*

Frequency (Hz)	E_θ (dynes/cm$^2 \times 10^6$)		E_z (dynes/cm$^2 \times 10^6$)		E_r (dynes/cm$^2 \times 10^6$)	
	E'_θ	E''_θ	E'_z	E''_z	E'_r	E''_r
0	6.12	0	9.51	0	5.07	0
0.5	6.87	0.09	14.38	1.80	5.49	0.41
1.0	6.91	0.07	14.78	1.70	5.59	0.41
2.0	6.91	0.20	15.23	1.30	5.66	0.22
3.0	7.08	0.08	15.09	2.49	5.80	0.60
4.0	7.15	0.13	15.05	1.86	5.81	0.46
5.0	6.96	0.02	14.66	2.44	6.19	0.66

E_θ, E_z and E_r are the complex incremental viscoelastic moduli in the θ, z and r directions; E'_θ, E'_z and E'_r are the real parts of the complex moduli representing the elastic coefficients and E''_θ, E''_z, and E''_r are the imaginary parts related to the viscous coefficients.

SUMMARY

We have developed an experimental technique which permits the quantification of the anisotropic, viscoelastic behavior of blood vessels *in vivo*. Most of the assumptions inherent to this technique have been experimentally justified. The viscoelastic properties of the MDTA and the elastic properties of LCCA and CA have been evaluated around a physiologic state of strain.

ACKNOWLEDGMENTS

We are grateful to Dr. D. L. Fry for his help and criticisms throughout this research. We thank Dr. T. E. Carew for his collaboration; Mrs. V. M. Fry for technical illustrations; Messrs. J. M. Pearce, F. Plowman, L. Brown and G. Johnson for technical assistance; Mrs. C. Floyd for data analysis; and Miss S. Green for editing and typing this manuscript. We also thank the American Heart Association, Inc., for permission to reproduce illustrations from previous publications.

REFERENCES

1. PATEL, D. J. and VAISHNAV, R. N. "Rheology of large blood vessels." *Cardiovascular Fluid Mechanics*. In press, Academic Press, London, 1972.
2. MCDONALD, D. A. *Blood Flow in Arteries*. Williams & Wilkins Co., Baltimore, 1960.
3. PATEL, D. J. and FRY, D. L. The elastic symmetry of arterial segments in dogs. Circ. Res. 24:1, 1969.
4. CAREW, T. E., VAISHNAV, R. N. and PATEL, D. J. Compressibility of arterial wall. Circ. Res. 23:61, 1968.
5. PATEL, D. J., JANICKI, J. S. and CAREW, T. E. Static anisotropic elastic properties of the aorta in living dogs. Circ. Res. 25:765, 1969.
6. PATEL, D. J. and JANICKI, J. S. Static elastic properties of the left coronary circumflex artery and the common carotid artery in dogs. Circ. Res. 27:149, 1970.
7. PATEL, D. J. and FRY, D. L. Longitudinal tethering of arteries in dogs. Circ. Res., 19:1011, 1966.

8. Vaishnav, R. N., Young, J. T., Janicki, J. S. and Patel, D. J. Non-linear anisotropic elastic properties of the canine aorta. Submitted, Biophys. J. 12: 1008–1027, 1972.

DISCUSSION

Perry L. Blackshear, University of Minnesota, Minneapolis, Minn.: How much of the property of the wall that the blood sees when it goes through a blood vessel is due to the blood vessel property and how much is due to its surrounding area?

Dr. Janicki: Are you referring to the vascular tethering?

Dr. Blackshear: Yes.

Dr. Janicki: Well, we could only calculate the contribution of tethering, and we found this to represent about 40 per cent of the longitudinal modulus we measured. When we removed it, we saw a 32 per cent decrease and this can be accounted for as the removal of this tethering tissue.

REQUIREMENTS FOR LONG-TERM CARDIOPULMONARY BYPASS CIRCULATION: HYPOTHESES AND DATA FROM RESEARCH ANIMALS

R. B. Shepard, E. I. McGowan, J. W. Scott, H. J. Baker, D. M. Bowen and K. M. Pruitt *

Methods for studies designed to separate the effects of protein denaturation from those of lack of normal pulsatile flow during cardiopulmonary bypass were developed. Hemodynamic energy carried by the pulsatile component of blood flow in various physiologic situations and during cardiopulmonary bypass was measured. Oxygen consumption in calves during bypass circulation with pulsatile blood flow of normal pulsatile energy content was found to be significantly greater than when flow was non-pulsatile. Blood and cerebrospinal fluid protein changes occurring in calves across the bypass period are described. In study of isolated and mixed gamma globulin and albumin protein fractions in a disc oxygenator, albumin was found to retard development of gross turbidity in mixtures of the two, and also to be present in precipitate which eventually formed. Human plasma from which gamma globulin and fibrinogen were removed did not develop precipitate. Mortality in calves given blood reconstituted from plasma oxygenated 20 hours was 60%. When Pluronic F-68 had been in the plasma through the oxygenation period, mortality was zero. A set of hypotheses regarding interaction between pulsatile flow and protein denaturation effects during cardiopulmonary bypass is proposed.

INTRODUCTION**

Total cardiopulmonary bypass circulation using pump oxygenators is extremely successful as now used clinically for an hour or two. Very long perfusions, however, are avoided, partially because of multiple organ system damage attributed to the perfusion process. No laboratory animal has been reported as a long-term survivor following twenty-four hours of total cardiopulmonary bypass. For long-term support of circulation alone, Bernhard and associates have described simultaneous biventricular pumping in normal calves for periods of 7 to 52 days, and total mechanical circulatory support for periods up to 32 hours.[1] For respiratory assistance alone, Kolobow and associates have reported partial extracorporeal blood gas exchange, using unanesthetized lambs, a spiral membrane lung, a veno-venous system, and a flow of 400 to 700 cc of blood per minute for one week with long-term survival, and for sixteen days with accidental death.[2] For cardiopulmonary bypass using peripheral arteriovenous cannulation and a bubble oxygenator, with induced ventricular fibrillation, Proctor and Parker have reported postoperative survival of unstated duration in some of twenty-five dogs kept on bypass for eight hours.[3]

The hypotheses proposed below concern *total cardiopulmonary* bypass circulation. Reasons for consideration of these hypotheses will become apparent, although the data are incomplete.

1. Blood flow with normal pulsatile hemodynamic energy content is necessary for normal whole-body oxygen consumption, and for prevention of problems related to tissue hypoxia, in long-term cardiopulmonary bypass.
2. Pulsatile flow is more lethal than steady flow when blood protein denaturation becomes appreciable during prolonged cardiopulmonary bypass circulation.
3. Particulate matter formation, and the deleterious physiologic effects of plasma protein denaturation, may be reduced markedly during prolonged cardiopulmonary bypass by using a surface active agent to change the gas-liquid interfacial forces acting on the plasma protein.

Experimental evaluation of these hypotheses will be examined in reverse order.

* The School of Medicine and The Medical Center, University of Alabama in Birmingham and the Veterans Administration Hospital, Birmingham, Alabama 35233.

** This work supported by National Heart and Lung Institute Grants H5884, H6167, HE 6353 and HE 11310. It was also supported in part by the Veterans Administration Medical and Surgical Services.

STUDY 1

The purpose was to determine the changes in blood and cerebrospinal fluid protein components which occurred as a result of cardiopulmonary bypass procedures in calves.[4] Calves were selected because of their convenient size for cardiovascular surgical research, their availability, and because sterile blood in 4,000 cc amounts for pump-oxygenator procedures could readily be obtained from individual, pregnant cows at a local dairy.

Methods, Study 1

Nineteen, approximately 3 month old calves were subjected to 4 hours of complete cardiopulmonary bypass circulation at 37°C. A standard twenty-five inch disc oxygenator, a roller pump, and a heat exchanger were used. For each procedure, the pump-oxygenator system was primed with a one-third hemodilution mixture. This consisted of 1250 cc of five percent dextrose in one-half normal saline, 360 cc of 1.4 percent sodium bicarbonate, thirty cc of ten percent calcium chloride, 36,000 units of heparin, and 2,000 cc of cross matched blood. For each calf, 4,000 cc of blood had been drawn, with sterile technique, from a single donor cow, into standard ACD bottles 24 to 48 hours prior to operation. An additional liter of balanced electrolyte solution or blood was added to the oxygenator during the procedure. Cardiopulmonary bypass, with the pulmonary artery clamped, was maintained at 2.1 L./min. per square meter of body surface area for 4 hours. Venous blood was taken from the right atrium and from the pulmonary artery proximal to the clamp. Arterial blood was returned to the aorta. Blood and cerebrospinal fluid samples were obtained from the animals before surgery, and after bypass at 2 hours, 48 hours, and 3–11 weeks. At these times, blood was collected from 19, 10, 6, and 4 animals, and cerebrospinal fluid from 16, 9, 5, and 2 animals, respectively.

Electrophoresis of serum and cerebrospinal fluid protein was performed using the Beckman Microzone System. The protein fraction percentages were determined densitometrically. Total serum protein was measured using the method described by Weichselbaum.[5] Cerebrospinal fluid total protein was determined by the procedure of Henry.[6] For evaluation of the electrophoretic patterns, control bovine serum and cerebrospinal fluid were not available. For cerebrospinal fluid, the scanned pattern of a human control sample was used as a guide.

Results, Study 1

The serum and cerebrospinal fluid values before and after operation are shown in Tables I and II. Data obtained from paired specimens are shown in Tables III and IV. Two hours after operation, serum total protein and the albumin and alpha globulin percentages had decreased while gamma globulin increased ($p < 0.02$). Beta globulin remained the same. During the same time, cerebrospinal fluid albumin increased and $alpha_1$ globulin decreased ($p < 0.05$).

STUDY 2

The purpose was to determine the behavior of isolated protein fractions in a disc oxygenator.[7] Gamma globulin and albumin were chosen because gamma globulin is easily denatured and albumin is often used as a stabilizer in protein solutions.

TABLE I.—*Serum Proteins of Calves Measured Before and After Total Cardiopulmonary Bypass Circulation of 4 Hours Duration with One-Third Hemodilution Prime*

	Before Operation	After cardiopulmonary bypass		
		2 hours	48 hours	3–11 weeks
Total Protein (gm/100 ml)	6.6 ± 0.5	5.3 ± 0.8	5.4 ± 0.3	8.1 ± 0.6
Albumin, percent	50.2 ± 4.5	47.4 ± 3.2	46.2 ± 6.0	27.2 ± 5.0
Alpha-globulin	15.4 ± 1.8	13.7 ± 1.0	19.7 ± 8.5	18.3 ± 1.0
Beta-globulin	14.3 ± 2.2	15.5 ± 4.5	11.2 ± 3.4	16.3 ± 3.3
Gamma-globulin	20.1 ± 5.6	23.4 ± 4.6	21.7 ± 6.4	38.2 ± 2.7
Number of Animals	19	10	6	4

Protein fractions determined by cellulose acetate electrophoresis and expressed as a percent of the total protein. Values expressed as mean ± standard deviation.

TABLE II.—*Cerebrospinal Fluid Proteins of Calves Measured Before and After Total Cardiopulmonary Bypass Circulation of 4 Hours Duration with One-Third Hemodilution Prime*

	Before Operation	After cardiopulmonary bypass		
		2 hours	48 hours	3–11 weeks
Total Protein (mg/100 ml)	30 ± 11	64 ± 51	78 ± 22	98 ± 65
Albumin*	44.5 ± 8.4	52.0 ± 7.2	53.9 ± 4.7	39.4 ± 0.6
Alpha1-globulin	14.0 ± 5.1	8.7 ± 2.3	9.3 ± 3.0	12.4 ± 2.9
Alpha2-globulin	11.3 ± 4.0	10.6 ± 2.4	9.9 ± 0.7	10.7 ± 1.8
Beta-globulin	17.6 ± 4.7	15.4 ± 2.7	14.3 ± 2.7	14.0 ± 0.1
Gamma-globulin	12.8 ± 5.1	13.3 ± 4.1	12.5 ± 1.6	23.4 ± 0.4
Number of Animals	16	9	5	2

* Protein fractions determined by cellulose acetate electrophoresis and expressed as a percent of the total protein. Values expressed as mean ± standard deviation.

TABLE III.—*Serum Proteins Before and After Total Cardiopulmonary Bypass Circulation of 4 Hours Duration with One-Third Hemodilution Prime. Paired Data from 10 Calves*

Serum	Before Operation	2 hours after bypass
Total Protein (gm/100 ml)	6.6 ± 0.4	5.3 ± 0.8*
Albumin, percent	51.3 ± 2.7	47.4 ± 3.3*
Alpha-globulin	15.8 ± 1.9	13.7 ± 1.1*
Beta-globulin	14.8 ± 1.9	15.5 ± 4.7
Gamma-globulin	18.1 ± 3.0	23.4 ± 4.8*

Protein fractions determined by cellulose acetate electrophoresis and expressed as a percent of the total protein. Values expressed as mean ± standard deviation.

* $p < 0.02$.

TABLE IV.—*Calf Cerebrospinal Fluid Proteins Before and After Total Cardiopulmonary Bypass Circulation of 4 Hours Duration with One-Third Hemodilution Prime. Paired Data from 9 Calves.*

Cerebrospinal Fluid	Before Operation	2 hours after bypass
Total Protein (mg/100 ml)	35.1 ± 10.6	64.1 ± 53.7
Albumin, percent	43.0 ± 10.8	52.0 ± 7.6*
Alpha1-globulin	11.9 ± 2.9	8.7 ± 2.4*
Alpha2-globulin	13.0 ± 4.0	10.6 ± 1.5
Beta-globulin	18.7 ± 5.3	15.4 ± 1.9
Gamma-globulin	13.4 ± 5.1	13.3 ± 4.4

Protein fractions determined by cellulose acetate electrophoresis and expressed as a percent of the total protein. Values expressed as mean ± standard deviation.

* $p < 0.05$.

Methods, Study 2

Purified gamma globulin and albumin were used.* Solutions were prepared in buffer of pH 7.4, total phosphate concentration 0.004 M, and sodium chloride concentration 0.14 M. Gamma globulin solutions were centrifuged for twenty minutes at 27,000 g in order to remove insoluble material before being placed in the oxygenator.

An otherwise standard disc oxygenator with its length shortened to 4 inches was used. The protein solutions were at room temperature, 23–26°C. Room air was in the gas section of the oxygenator; it was not circulated. The number of revolutions of the discs was counted electromechanically. Total interfacial gas-liquid surface area produced during a given time was calculated using the area of the discs which became immersed in liquid during each revolution, and the total number of revolutions during the time period.

Relative turbidity was measured in a Brice-Phoenix light scattering photometer model 2000 held at 25°C. The light source was a mercury lamp fitted with a monochromatic filter with a 436 mμ peak. Intensity of scattered light was measured at 90°.

Fluorescence spectra were recorded with an Aminco-Bowman spectrophotofluorometer. The fluorescent probe 1-anilinonapthalene-8-sulfonate (ANS) was purified by recrystallization and was used for the detection of albumin in the presence of gamma globulin.[7] Extrinsic fluorescence was measured by mixing an excess of the dye with protein solutions and measuring emission at 465 ± 10 mμ (excitation at 375 ± 10 mμ).

Results, Study 2

Gamma globulin solution turbidity development was nearly linearly related to the total interfacial area exposure, up to the point of precipitation of the denatured protein. The turbidity which developed was a direct function of the initial gamma globulin concentration. The maximum relative turbidity was 25 times higher than the initial value. When albumin, 1.6 mg/ml, was in solution with gamma globulin 0.4 mg/ml,

* Obtained from the Pentex Corporation.

turbidity development was inhibited by an order of magnitude. When albumin solution alone was in the oxygenator, small but rapid increases in relative turbidity by a factor of 1.2–1.3 occurred.

The preparations of albumin used were soluble in solutions approximately 75% saturated with ammonium sulfate. This solubility of albumin alone was not altered by the oxygenator. The gamma globulin alone was completely precipitated by this concentration of ammonium sulfate; the gamma globulin was soluble in solutions approximately 30% saturated with ammonium sulfate. Gamma globulin which had been in the oxygenator showed a decrease in solubility in 30% ammonium sulfate solution. This decrease was related to the magnitude of the gas-liquid interfacial area exposure which had occurred.

Albumin-globulin mixtures exposed to the oxygenator showed solubility properties intermediate between those of the individual proteins. The change in solubility properties which occurred in the mixtures took place within the first few minutes of oxygenator operation, as did the occurrence of small increases in turbidity of albumin alone. In the mixtures which had been in the oxygenator, albumin was found in both the supernatant and the precipitate which were produced by exposure to ammonium sulfate solution.

These data were interpreted as indicating that the gas-liquid interfacial exposure of mixtures of albumin and gamma globulin in the oxygenator resulted in production of a protein species which was not produced when either solution alone was in the oxygenator. The data show that this produced species contained both albumin and gamma globulin. Additional interpretation is that the first step in protein denaturation which occurred in these experiments was unfolding of the gamma globulin molecule in the liquid-gas interface, and that a second step was the interaction of the unfolded molecule with another gamma globulin molecule. The latter molecule could have been either native or altered. In subsequent steps, unfolded gamma globulin molecules interacted with other aggregates to produce the observed large increases in turbidity and, ultimately, precipitation. The inhibition of turbidity development by an order of magnitude when albumin was present with gamma globulin may be interpreted as indicating that denatured gamma globulin molecules interacted with albumin to produce a complex which did not aggregate further. Such a complex could account for the altered solubility properties in ammonium sulfate which were observed after the mixtures of albumin and gamma globulin were exposed in the oxygenator.

STUDY 3

The purpose was to determine what presently available methods, with potential clinical usefulness, could be utilized to decrease protein denaturation in the disc oxygenator. Making changes in the oxygenator oxygen tension, and changing the plasma protein initial composition, were considered, the former because of the work of Ashmore and associates,[8] and the latter because of the results with plasma protein fractions reported above. In addition, evaluation of a chemical with both hydrophobic and hydrophillic properties, which conceivably could reduce the interfacial forces acting on the globulin molecule at the gas-liquid junction in the oxygenator, was considered. Nine, gross screening procedures were carried out. The quantity of particulate matter which collected in the bubble trap of the pump-oxygenator system when plasma was oxygenated twenty hours was observed. This information was later used as a guide for additional procedures, with calves.

Methods, Study 3

Bovine plasma was used five times. In each instance, 4,000 cc of blood was drawn from a pregnant donor cow into standard ACD bags, using sterile technique. The blood was centrifuged for ten minutes at 5°C at 4,200 RPM and the plasma was removed.

A standard disc oxygenator, roller pump, heat exchanger, bubble trap, and venous reservoir system was set-up for each procedure. Clinical grade Tygon tubing was used. The arterial and venous lines from the pump-oxygenator system were connected together to form a sterile, closed circuit. One thousand cc of 5% dextrose solution in one-half normal saline, 325

cc of pH 7.4 balanced electrolyte solution, 360 cc of 1.4% sodium bicarbonate, 36,000 units of heparin, and 2,000 cc of cow plasma were put in the system. Oxygen and carbon dioxide for the oxygenator were filtered through a 0.22μ Millipore filter. Initial gas flow through the oxygenator was 5 L/min; initial added carbon dioxide flow was 100 cc/min. One-hundred and thirty-two discs were in the oxygenator. They were rotated at 100 RPM. The discs had been siliconized prior to autoclaving and assembly of the oxygenator. Carbon dioxide flow into the oxygenator was adjusted to maintain the plasma pH at 7.4. Plasma carbon dioxide tension and oxygen tension were measured 3 or more times during each procedure. The roller pump was run to circulate the plasma solution through the oxygenator at 1900 ± 250* cc per minute. Sterile technique was used throughout the twenty-hour circulation period.

For one bovine plasma procedure, the polyoxyethylene-polyoxypropylene compound Pluronic F–68** in flake form was weighed, autoclaved, dissolved in distilled water in a concentration of 3.33 gm.%, and was added to the fluid in the pump-oxygenator to produce a concentration of 0.6 mg. of Pluronic F–68 per cc of total system volume. In other procedures, nitrogen was used instead of oxygen once, and room air instead of oxygen on another occasion. Pluronic was not used in either of these procedures.

In three additional procedures, human plasma was used in place of cow plasma, once with the disc oxygenator, once with a bubble oxygenator with a filter*** and once with the disc oxygenator with Pluronic solution added. Human plasma from which the fibrinogen and gamma globulin had been removed† was used once. In those procedures involving human plasma, the solution volumes were half those specified for the bovine plasma procedures, and a pediatric-size oxygenator was used.

* Mean ± standard deviation.

** Furnished initially by the Wyandotte Chemicals Corporation, Wyandotte, Michigan, and later by Cutter Laboratories. Berkeley, California.

*** Furnished by Bentley Laboratories, Inc.

† Cutter Plasmanate, furnished by the Birmingham Regional Red Cross Blood Center.

TABLE V.—*Results of Oxygenator Circulation*

Results after 20 hours circulation	Plasma Bacterial Cultures*	Turbidity	Particulate Matter in Bubble Trap
Bovine plasma and disc oxygenator			
With air	Negative	++	++
With nitrogen	Negative	+++	++++**
With oxygen	Negative	+++	++++**
With oxygen and Pluronic	Positive	+	–
Human plasma and disc oxygenator			
With oxygen	Not done	++	+++
With oxygen and Pluronic	Negative	+	–
Human plasma with gamma-globulin and fibrinogen removed; disc oxygenator	Positive	+	–
Human plasma and bubble oxygenator with blood filter	Positive	+	–

*Drawn after plasma had been in oxygenator 20 hours.

**An inch and a half of precipitate was present over half the diameter of the bubble trap.

Results, Study 3

Gross amounts of particulate matter were found to have settled in the bubble trap in all instances except when (1) human plasma free of gamma globulin and fibrinogen had been used, (2) human or bovine plasma with Pluronic F–68 had been used, or (3) the bubble oxygenator with its filter had been used (Table V). In the bubble oxygenator, much particulate matter was found in the filter.

Plasma pH during these procedures was 7.41 ± .16 (n = 30), and the carbon dioxide tension was 37 ± 17 mm Hg (n = 31). When nitrogen was used instead of oxygen, plasma oxygen tension was 53 ± 17 mm Hg. When air was used, oxygen tension was 133 ± 4 mm Hg. Oxygen tension in the plasma in those procedures using oxygen was 539 ± 21 mm Hg.

Using fluorescence of ANS as an indicator, albumin was found present in the particulate matter removed from the bubble trap.

STUDY 4

Methods, Study 4

The purpose was to compare lethal effects in calves of plasma oxygenated 20 hours with and without Pluronic F–68 being present during the period of oxygenation.

For each of 16 procedures, 4,000 cc of blood were drawn from a pregnant donor cow which had been cross matched with an approximately 70 kg calf. The blood was separated into cells and plasma as described for Study 3. The plasma

was then put in the oxygenator with the same prime solution described for Study 3. The oxygenator had 132 discs; these were rotated at 100 RPM. Oxygen and CO_2 entering the oxygenator were filtered through a 0.22 μ Millipore filter. Plasma pH was controlled by changing the CO_2 flow into the oxygenator. The plasma was circulated through the oxygenator at 2,300 ± 575 cc/min. It was kept at 37°C. After 20 hours, the red cells were returned to the plasma and mixed with it in the pump oxygenator circuit. Calcium chloride, 10% solution, 30 cc was added.

Each calf was healthy as judged by observation. Each received penicillin (500,000 units) and streptomycin (1 gram) 24 hours before operation and the morning of operation. Anesthesia was induced with 4% halothane, given through a plastic bag fitted over the nose. An endotrachael tube was inserted. Anesthesia was maintained with 0.5 to 1.5% halothane and 50% oxygen—50% nitrous oxide gas flow. A Bird mechanical ventilator was used with the anesthesia machine. The left external jugular vein and left common carotid artery were exposed. Heparin, 150 units/kg, was given 5 minutes before insertion of a cannula through the external jugular vein into the right atrium. The left carotid artery was occluded with a vascular clamp and a #12–#16 arterial perfusion cannula was inserted in the proximal portion. Right atrial pressure was measured through a central venous catheter. Arterial pressure was measured through a second small catheter placed, using the Seldinger technique, through the left common carotid artery well into the aorta. Strain gage transducers were used.

Venous flow to the pump oxygenator was controlled initially to keep the right atrial pressure at the level existing prior to bypass. If arterial blood pressure fell to less than 90 mm Hg, the calf's blood volume was increased until venous pressure reached 12 mm Hg, or until systolic pressure was above 90. If right atrial pressure were 12 mm Hg and systolic pressure remained less than 90, epinephrine in 1/500,000 concentration was given intravenously at a rate adequate to restore the systolic pressure to 90 within five minutes.

Oxygenated blood flow from the pump to the calf was 0.5 to 2.1 L/min/M^2. It was continued for 16–30 minutes, until 95% or more calculated mixing of the blood in the pump oxygenator and in the animal had occurred. The perfusion cannulas were then withdrawn and the carotid artery cannulation site was accurately repaired with 5–0 synthetic vascular suture. The neck wound was closed and the calf was held upright in a sling while recovering from anesthesia. Respiratory assistance with a ventilator and endotrachael tube was continued when necessary. Surviving calves were later returned to pens for continued periodic observation.

During the procedures, cultures for bacterial growth were obtained from donor whole blood, plasma, plasma and prime solutions mixed, blood after the red cells had been returned, blood in the oxygenator at the end of bypass, and blood from the calf at the end of the procedure. Samples for determination of pH, PCO_2, PO_2, hematocrit and hemoglobin oxygen saturation were taken periodically.

In 8 of the 16 procedures described, Pluronic F–68 was added at the time the plasma was put in the oxygenator, to produce a concentration of 0.6 mg/ml. No Pluronic was used in the other eight procedures.

Two control procedures were carried out initially. In one, the left common carotid artery of a heparinized calf was clamped for 12 minutes. In the other the entire procedure as described above was done except that the 20-hour period of oxygenation was omitted.

Results, Study 4

Immediately before bypass, in the non-Pluronic and Pluronic groups respectively, systolic pressures were 129 ± 27 and 133 ± 25 mm Hg, diastolic pressures were 93 ± 22 and 98 ± 24 mm Hg, and right atrial pressures were 4 ± 4 and 2 ± 5 mm Hg. None of these differences were significant.

A significant decrease in blood pressure occurred after 16 minutes of partial bypass in the non-Pluronic group compared to the Pluronic group. Systolic pressure was 54 ± 8 in the former compared to 97 ± 15 in the latter ($p < 0.05$). Diastolic pressures were 38 ± 7 and 77 ± 14, respectively ($p < 0.05$). At this time, right atrial pressures were 4 ± 6 mm Hg in the non-

Pluronic group and 1 ± 4 mm Hg in the Pluronic group.

Eight minutes after beginning partial bypass, systolic pressure in the non-Pluronic group was 94 ± 14, while in the Pluronic group it was 70 ± 10. Diastolic pressures were then 73 ± 12 and 47 ± 11, respectively, and right atrial pressures were 3 ± 5 mm Hg in both groups.

Systolic pressure was returned to 90 mm Hg or above by blood infusion from the pump, with guidelines as described above, when it decreased during these episodes.

When bypass was stopped, two calves of the Pluronic group sustained systolic pressure drops to 60 and 55 mm Hg, while right atrial pressure increased. This depression in ventricular function was definite, but transient as judged by duration of need for epinephrine infusion. Pulmonary edema was present in one of these animals.

Immediately following operation, while the calves were being ventilated with an oxygen-driven Bird respirator, arterial blood gas oxygen tensions were 194 ± 115 in the non-Pluronic group and 181 ± 92 mm Hg in the Pluronic group. Carbon dioxide tensions were 30 ± 3 and 32 ± 8 mm Hg, and pH values were 7.48 ± .01 and 7.45 ± .13, respectively.

Five of the 8 non-Pluronic group of calves died within 24 hours. They failed to regain consciousness fully and initially had convulsive movements of the extremities. The 3 animals which survived longer than 24 hours showed extension of the neck and legs consistent with decerebrate rigidity.

All of the 8 calves in the Pluronic group and both control animals were free of any gross evidence of neurologic damage, and all were long term survivors.

Bacterial culture results are shown in Tables VI and VII. These are interpreted as showing no bacterial influence on the results reported.

STUDIES 5 AND 6

The purpose of Study 5 was to describe, in quantitative terms, the energy characteristics of pulsatile blood flow in a variety of surgical and pathophysiologic situations.[9] It was found that pulsatile blood flow varies markedly in its pulsatile energy characteristics, according to the physiologic situation, but that it normally carries more hemodynamic energy per cc of blood passing through a given arterial cross-section than does non-pulsatile flow with the same mean pressure. This difference can be expressed as the difference between an *energy equivalent pressure* and mean arterial blood pressure in mm Hg, or it can be expressed directly as an energy difference between pulsatile and non-pulsatile flow in the ergs/cc of blood flowing through the artery. Accurate phasic flow and pressure data are necessary for the computation. In dogs undergoing cardio-

TABLE VI.—*Culture Data*

Numerical Code for Type of Contamination:
1. Grossly contaminated—not identified
2. Contaminated—not identified
3. Micrococcus sp.
4. Pseudomonas sp.
5. Gaffkya sp.
6. Diptheroid
7. Strep viridans
8. Mold
9. Proteus sp.
10. Many different gram(-) rods. Lactose positive
11. Omitted

Blank spaces indicate negative cultures.

	Non-Pluronic Calves							
	#1	#2	#3	#8	#9	#10	#11	#15
Donor whole blood				3				11
Plasma from collection bag								11
Plasma and prime mixed			5	10				11
Reconstituted blood					6	3		11
Blood from pump after bypass								11
Oxygen filter after bypass	2				8			11
Blood from calf after bypass					4	9		11

TABLE VII.—*Culture Data*

Numerical Code for Type of Contamination:

1. Grossly contaminated—not identified
2. Contaminated—not identified
3. Micrococcus sp.
4. Pseudomonas sp.
5. Gaffkya sp.
6. Diptheroid
7. Strep. viridans
8. Mold
9. Proteus sp.
10. Many different gram(-) rods. Lactose positive
11. Omitted

Blank spaces indicate negative cultures.

	Pluronic Calves							
	#4	#5	#6	#7	#12	#13	#14	#16
Donor whole blood				8			11	11
Plasma from collection bag						8	11	11
Plasma and prime mixed	11						11	11
Reconstituted blood	11	3	1	3			11	11
Blood from pump after bypass	11	3					11	11
Oxygen filter after bypass	11		1		7		11	11
Blood from calf after bypass	11			3			11	11

pulmonary bypass circulation with a roller pump, energy equivalent pressure and mean pressure in the thoracic aorta during bypass were identical within the range of experimental error. Before and after bypass, energy equivalent pressure was greater than mean pressure.

Study 6 was designed to determine if whole body physiologic effects occurred as a result of the presence or absence of the pulsatile component of total hemodynamic energy.[10] It was carried out in 23 calves subjected to 4 hours of total cardiopulmonary bypass at 2.1 L/min/M^2. Ten animals were perfused with pulsatile flow designed to have normal pulsatile energy characteristics. This was introduced, to avoid losses in the pulsatile component of total hemodynamic energy, through a large graft sewn on the side of the aorta. In these calves, the pulsatile energy component of total hemodynamic energy was 10,000 $\pm$ 5,000 ergs/cc during operation prior to bypass, 10,600 $\pm$ 2,700 ergs/cc during bypass and 440 $\pm$ 470 during non-pulsatile bypass ($p < .001$). Oxygen consumption measured at hourly intervals in the animals perfused with pulsatile flow in this manner was 126 $\pm$ 18 cc/min/M^2. In 13 calves perfused similarly with non-pulsatile flow, oxygen consumption was 101 $\pm$ 17 cc/min/M^2. ($p < .005$). A vent line in the left ventricle decompressed the left atrium and ventricle in both groups. Bronchial artery flow, as judged by volume of left ventricular vent drainage when the pulmonary artery was occluded, was 128 $\pm$ 56 cc/min/M^2 in the pulsatile group and 36 $\pm$ 11 cc/min/M^2 in the non-pulsatile group ($p < .01$). Catecholamines were not significantly different between groups. All of the non-pulsatile group of 13 calves and 4 of the pulsatile group of 10 were long-term survivors. Post bypass pulmonary dysfunction was prominent in the pulsatile group.

These data are interpreted as indicating that an increase in oxygen consumption and change in regional distribution of blood flow occurred when perfusion was properly pulsatile compared to when it was non-pulsatile. The data are also interpreted as probably indicating that increased perfusion of the bronchial arterial system, in the pulsatile group, distributed increasingly large amounts of denatured plasma protein through the bronchial circulation, leading to the pulmonary dysfunction observed after operation.

STUDY 7

The purpose is to use the information described above to accomplish complete cardiopulmonary bypass circulation in calves for 24 hours with long-term survival. The study is incomplete. Using pulsatile flow and a disc oxygenator with Pluronic F–68 added, and other techniques as described above, two calves have been maintained on complete bypass for 20

hours. One died 48 hours after operation with laryngeal edema. Weight gain was 3 pounds. The other died 6 hours after bypass was completed, the immediate cause being inadequate replacement of post-bypass chest tube drainage. Both animals were awake. Each moved normally and each had greater than 95% arterial blood oxygen saturation when breathing room air after operation.

DISCUSSION

Pluronic F–68 was found to diminish occurrence of systemic fat emboli in cardiopulmonary bypass circulation, by Adams and associates, in 1959.[11] A moderate-size literature about its use has developed.[12] The pathophysiology of cardiopulmonary bypass circulation has been widely studied.[13]

The data described in this paper indicate that during cardiopulmonary bypass circulation, pathophysiologic effects occurring due to lack of normally pulsatile arterial blood flow, and due to protein denaturation, may often be confounded. The data presented, obtained in an effort to separate these effects, support the hypotheses proposed in the introduction to this paper.

SUMMARY

Gamma globulin and albumin put in a disc oxygenator produced a precipitate which was a species of denatured gamma globulin and albumin combined.

Both bovine and human plasma developed gross amounts of precipitate during 20 hours in a disc oxygenator. The precipitate did contain albumin.

Human plasma from which gamma globulin and fibrinogen had been removed did not form a precipitate during 20 hours in a disc oxygenator.

Neither bovine nor human plasma formed gross precipitate when Pluronic F–68 had been added to the plasma prior to the 20 hour period in the disc oxygenator.

When 8 calves were subjected to brief veno-arterial, partial bypass circulation, with blood reconstituted from plasma which had been in a disc oxygenator 20 hours, 5 died. All had profound neurologic damage.

Under similar circumstances, but with Pluronic F–68 added to the plasma prior to the period of oxygenation, all of 8 calves were long-term survivors, without neurologic damage, as judged by observation.

Oxygen consumption in 23 calves maintained on cardiopulmonary bypass circulation for 4 hours was 101 ± 17 cc/min/M^2 in 13 animals perfused with non-pulsatile flow, and 126 ± 18 cc/min/M^2 in 10 animals perfused with pulsatile flow of normal pulsatile energy characteristics.

Two calves have been maintained on complete cardiopulmonary bypass for 20 hours. Pluronic F–68 was added to the blood in the disc oxygenator and pulsatile flow was used. Death occurred 48 and 6 hours after operation, respectively. Post-operative technical problems were the immediate causes of death.

For comparison of studies involving response to pulsatile blood flow, quantitative statement of pulsatile energy characteristics of the flow in the animal rather than in the pump is necessary.

ACKNOWLEDGMENTS

The authors are grateful, for their advice or work with these projects, to R. M. Stroud, T. A. Noto, J. E. Bearden, E. H. Bell, L. V. Branham, M. T. Brooks, J. A. Burdeshaw, G. E. Carpenter, G. H. Cassell, C. R. Cooper, F. E. Harrell, L. N. Larkin, B. K. Phelps, C. A. Reaves, S. H. Redmond, J. L. Schaeffer, J. F. Sharp, D. C. Simpson, A. F. Spear, E. J. Stephenson, J. L. Stewart, E. M. Vaughn, M. S. Waits, J. C. Walker, C. L. Wallace, J. C. Wells, and H. A. Wingate, Jr., over a 10-year period.

REFERENCES

1. Bernard, W. F., LaFarge, C. G., Bankole, M., Bornhorst, W., and Button, L. Biventricular bypass: Physiologic studies during induced ventricular failure and fibrillation. J. Thor. & Cardio. Surg. 62:859–868, 1971.
2. Kolobow, T., Spragg, R. G., Pierce, E., and Zapol, W. M. Extended term (to 16 days) partial extracorporeal blood gas exchange with the spiral membrane lune in unanesthetized lambs. Transactions,

American Society For Artificial Internal Organs, XVIII: 350–354, 1971.
3. Proctor, E., and Parker, R. Total circulatory support by peripheral cannulation and pump oxygenation during 8 hours of ventricular fibrillation in the dog. J. Thor. & C. V. Surg. 57:702–707, 1969.
4. McGowan, E. I., and Noto, T. A. The effect of extracorporeal circulation on calves' blood and cerebrospinal fluid proteins. Proceedings of the 12th Congress of the International Society of Blood Transfusion, Moscow, 1969; Karger AG, Arnold-Bocklin-Strasse, 25, CH–4000 Basel II (Switzerland).
5. Weichselbaum, T. E. Accurate and rapid method for determination of proteins in small amounts of blood serum and plasma. Amer. J. Clin. Path. 10:40–49, 1946.
6. Henry, R. J., et al. Turbidimetric determination of proteins with sulfosalicyclic and trichloroacetic acids. Proc. Soc. Exp. Biol. Med. 92:748–751, 1956.
7. Pruitt, K. M., Stroud, R. M., and Scott, J. W. Blood damage in the heart-lung machine. Proc. Soc. Exper. Bio. & Med. 137:714–718, 1971.
8. Ashmore, P. G., Svitek, V., and Ambrose, P. The incidence and effects of particulate aggregation and microembolism in pump-oxygenator systems. J. Thor. & Cardio. Surg. 55:691–697, 1968.
9. Shepard, R. B., Simpson, D. C., and Sharp, J. F. Energy equivalent pressure. Arch. Surg. 93:730–740, 1966.
10. Shepard, R. B., and Kirklin, J. W. Relation of pulsatile flow to oxygen consumption and other variables during cardiopulmonary bypass. J. Thor. & Cardio. Surg. 58:694–702, 718–720, 1969.
11. Adams, J. E., Owens, F., Mann, G., Headrick, J. R., Munoz, A., and Scott, H. W. Experimental evaluation of pluronic F68 (a non-ionic detergent) as a method of diminishing systemic fat emboli resulting from prolonged cardiopulmonary bypass. Surg. Forum 10:585–589, 1959.
12. Danielson, G. K., Dubilier, L. D., and Bryant, L. R. Use of pluronic F–68 to diminish fat emboli and hemolysis during cardiopulmonary bypass. J. Thor. & Cardio. Surg. 59:178–184, 1970.
13. Peirce, E. C. *Extracorporeal Circulation For Open-Heart Surgery.* Chapter 2. Charles C. Thomas, 1969.

DISCUSSION

Franklin G. Robinson, Cutter Lab., Berkeley, California: What dosage of Pluronic was used?

Dr. Shepard: This was the older Pluronic obtained as flakes from the Wyandotte Corporation. It was dissolved in distilled water at a concentration of 3.3 grams per cent, and the contents were diluted in the oxygenator so that the final concentration was .6 of a milligram per milliliter, which is the same concentration reported in the literature. As you know, there's a lot of literature about the use of Pluronic. Dr. Lester Bryant from Kentucky, who is attending this conference, has published a paper about its clinical use.

Dr. Robinson: Is that with or without Ironal?

Dr. Shepard: The material was furnished simply as Pluronic F-68. A preservative may have been present. If so, no statement about its presence was made to us by the manufacturer.

Jeffrey L. Peters, University of Utah, Salt Lake City, Utah: How were the red cells handled during this twenty-hour period?

Dr. Shepard: The red cells during the period of oxygenation of the plasma were refrigerated in plastic bags. They had been spun down and maintained at 4°C.

HEMODYNAMICS IN THE CHIMPANZEE: UNANESTHETIZED AND ANESTHETIZED

H. L. Stone* and H. Sandler**

The cardiovascular system of the chimpanzee has not been studied very extensively, and this animal's relationship to human standards in this regard are not well known. The response of this animal to various common stimuli must be known in order to propose the chimpanzee as an analogue of man or for human disease involving the cardiovascular system. Seven chimpanzees weighing 23 to 32 kg were used in this study. A left thoracotomy was performed in the fourth intercostal space under sterile surgical conditions. A multichannel telemetry unit containing ECG electrodes and 3 to 6 solid state pressure cells was implanted in each animal. Pressure cells were implanted in the right and left ventricular cavities and the left atrium. The remaining pressure cells were left in various locations in the thorax. An electromagnetic or Doppler ultrasonic flow probe was implanted around the ascending aorta. The chest was closed and evacuated. Telemetry signals were recorded periodically from the unrestrained caged animals following surgery. The response of these animals was determined to anesthesia, norepinephrine and isoproterenol. Results indicate that the chimpanzee has an elevated mean arterial pressure by human standards and that the response pattern to most conditions is mediated by the sympathetic nervous system.

INTRODUCTION***

Interest in the study of various non-human primates has increased over the past 15 years as demonstrations have been made that they may serve as realistic models for various physiologic processes in man. Such animals are phylogenetically and anthropomorphically close to man, have a near human posture and are highly intelligent. The chimpanzee of all non-human primates has been thought to be closest to man.[1] Chimpanzees have been used extensively in neurophysiologic[2] and social behavioral studies,[3] are susceptible to a wide variety of human infections[4] and were chosen by the Space Agency in the late 1950's as the best animal model to simulate man prior to space flight.[5]

Although a chimpanzee's heart was used as donor for the first human heart transplantation,[6] only a few cardiovascular studies and demonstrations of cardiovascular function have been reported in these animals.[7-13] The first studies of the systemic and pulmonary circulations of the chimpanzee were reported by Weissler in 1963.[8] These latter studies were conducted using standard clinical catheterization techniques in awake but restrained animals. Heart rates and direct recordings of arterial pressures were significantly elevated by human standards. These findings correlated with previous observations reported by the Space Agency using similar animal preparations as part of ground based observations prior to space flight.[5] On the basis of these previous findings and with the development and use of multichannel implantable telemetry units in these laboratories, it was decided to obtain additional blood pressure recordings in the chimpanzee in an awake unrestrained state and to determine cardiovascular response to anesthesia and standard sympathomimetic drugs.

METHODS

Data were collected on 7 animals. All animals were obtained from the 6571st Aeromedical Research Laboratory, Holloman Air Force Base, Allamagordo, New Mexico; five were used for purposes of chronic instrumentation. They consisted of 3 males and 2 females ranging in age from 5 years to 7 years. Two uninstrumented animals in this same age span were also included as controls. The smallest animal weighed 23 kg and the largest weighed 32 kg with an average weight of 28 kg. The study was conducted in two parts, separated by a year. Two animals

* The Marine Biological Institute Galveston, Texas.
** NASA-Ames Research Center Moffett Field, California.
*** Supported in part by NASA Contract No. NGR-44-088-002.

were instrumented in February 1970 (Group I), three animals were instrumented in March 1971 (Group II). The two uninstrumented animals were studied with the Group I animals.

Eight-channel telemetry units were implanted in both Group I instrumented animals; two 5-channel units and an 8-channel unit were implanted in the Group II animals. The operation of such multichannel units has been previously described.[14,15] Multichannel operation is provided by a time sharing multiplex scheme for switching between the various sensor inputs; data are transmitted on a single carrier frequency using pulse interval modulation. One data channel in each unit is used to transmit a long duration pulse from a reference bridge to provide synchronization for the multiplex scheme. Battery power is conserved by the use of an implantable radio-frequency actuated switch. The manner of recording data from each animal in the immediate laboratory environment is schematically illustrated in Figure 1.

A standard squeeze cage was utilized to house all animals. The perching bar of the cage was modified to serve as the receiving antenna for the implanted units. The movable wall of each

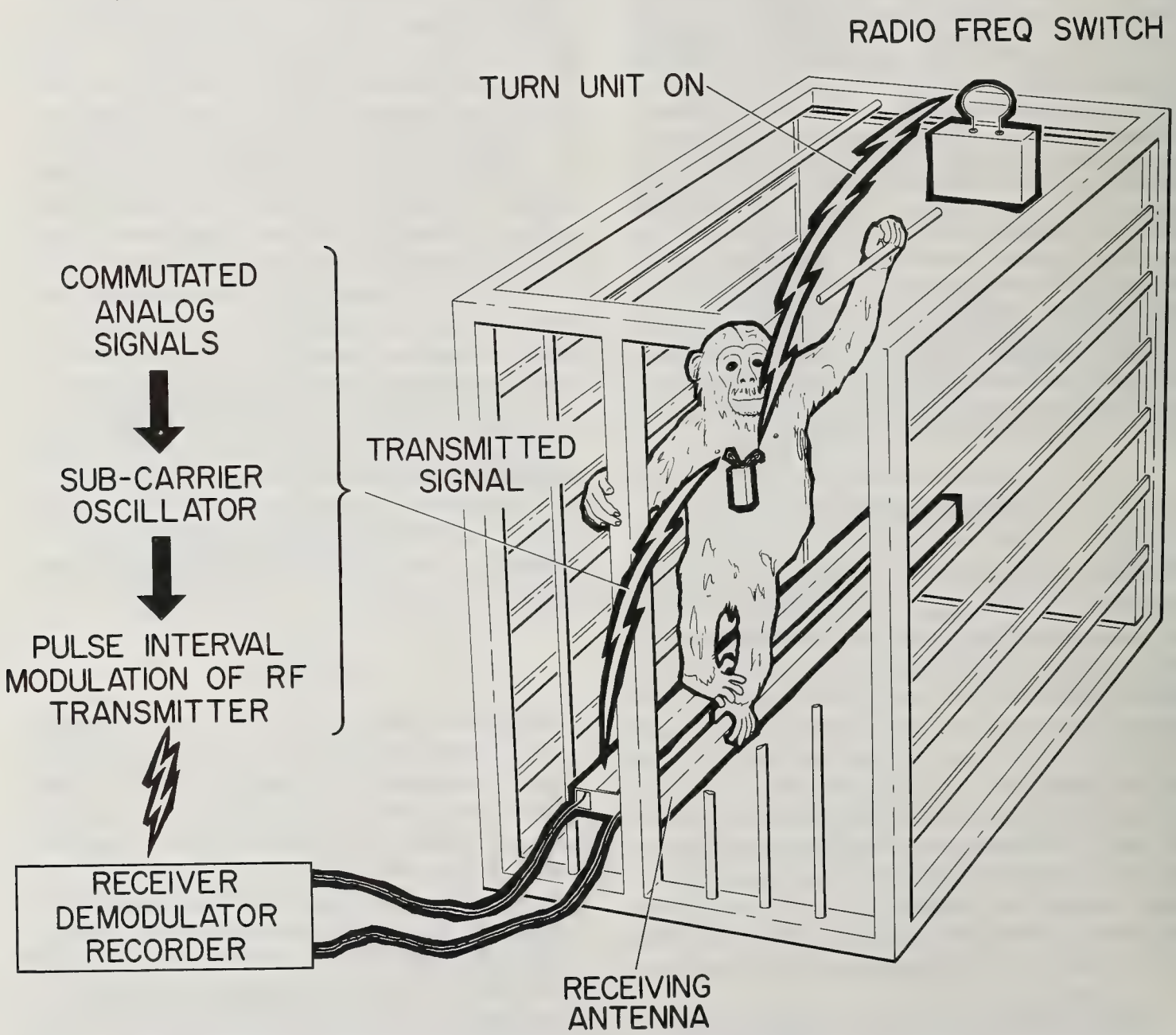


FIGURE 1.—Manner of obtaining cardiovascular data by telemetry from a chimpanzee.

cage contained the actuating switch for the implanted units enclosed in a plastic protected shield; actuation was accomplished by manual or remote means. The implanted unit is powered by two pacemaker cells providing 1000 milliampere hours of power which allows for 500 hours of continuous operation of the unit. With intermittent sampling it is anticipated that 1–2 years of data can be obtained from appropriately instrumented animals. One such implanted unit has now operated with intermittent samplings for 11 months and continues to operate at the time of this writing. All data was recorded on an Ampex FR 1300 tape recorder for subsequent analysis or display.

The manner of calibration of the implanted units has been previously described.[15] Units were carefully bench calibrated prior to implantation and by direct and indirect means while within the chest cavity of each respective animal. ECG was measured in all animals. Three pressures were recorded in two animals, six pressures were recorded in one animal, four pressures and two single axis accelerometers in two animals and three pressures and one three axis accelerometer in the last two animals. Accelerometers were used as dimensional gages and to study the vibrational characteristics of the left ventricular wall.[16,17] Pressure cells were implanted in the right and left ventricles and left atrium in all animals. Figure 2 shows a lateral chest x-ray in one animal illustrating the placement of the transmitter and various transducers. Additional cells were left in the apical

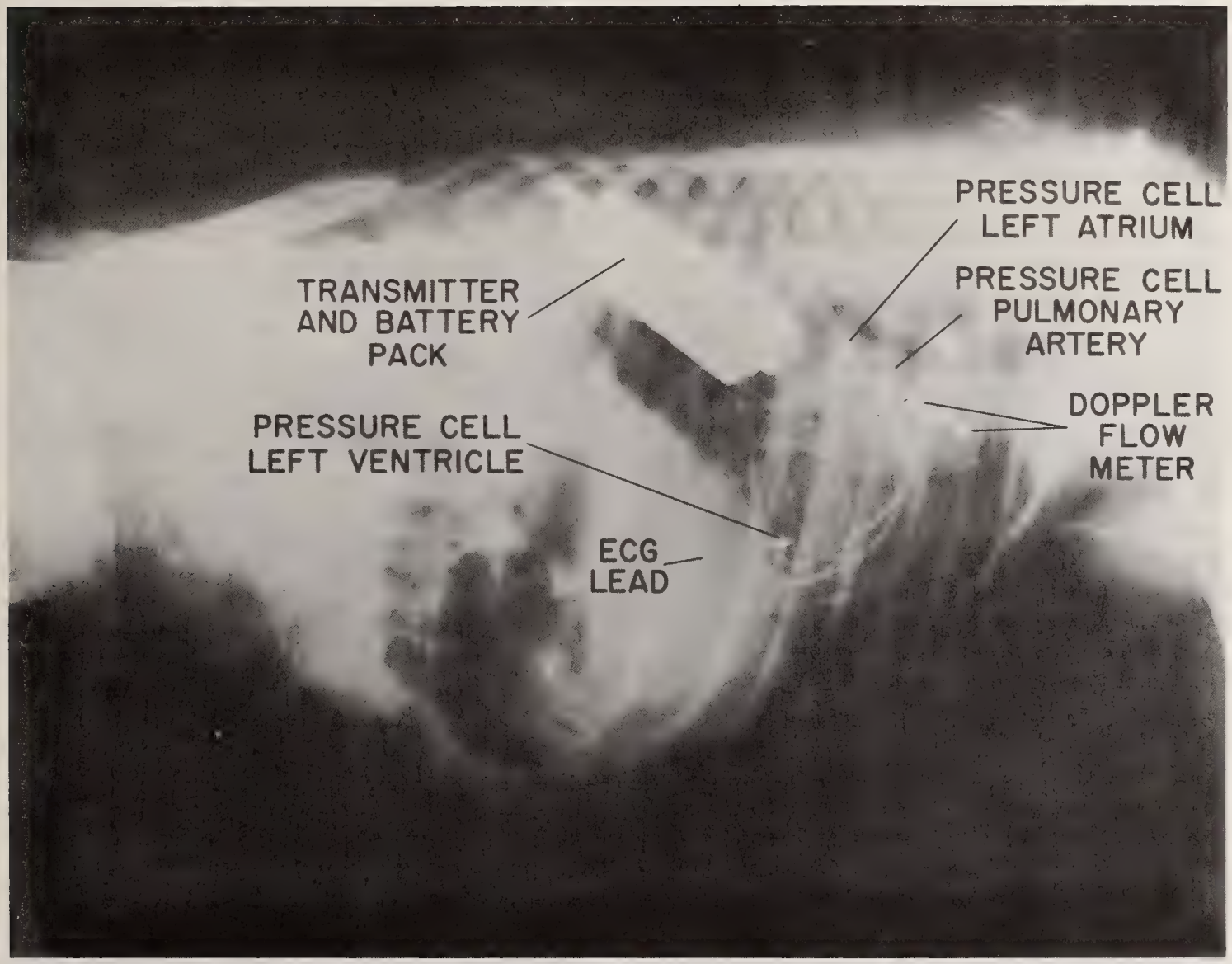


FIGURE 2.—Lateral chest radiograph in a chimpanzee.

or basal portions of the left pleural space when using units containing more than three pressure cells. The pre- and post-operative procedures for implanting these devices has been previously described.[18] All animals in Group I were additionally instrumented with electromagnetic flow meters placed on the ascending aorta; these were replaced with Doppler ultrasonic trandsucers in Group II animals. Group I animals were additionally studied by Doppler flow meters placed on the common carotid and subclavian arteries as previously described.[18] Lead wires from all flow transducers were buried beneath the skin in the vicinity of the original thoracotomy incision and exteriorized following anesthesia at the time of subsequent studies.

Four animals were actually instrumented in the Group I study but two died 18 and 37 days post-operatively from pneumonia. The third animal in this group succumbed from an empyema and sepsis subsequent to replacement of the implanted battery pack 3 months after surgery. Change in antibiotic regime has subsequently remedied this situation. The last animal has survived without complications and is now 23 months post-surgery and has gained 25 pounds during this period. The implanted units operated for only two weeks in each animal in Group I. Examination of the units subsequent to removal at post-mortem revealed leakage of body fluids into the site of union of transducer leads with the transmitter and corrosion of solder joints in that area.

All animals in Group I (two instrumented, two uninstrumented) were studied only after intramuscular anesthesia using 0.8 mgm/kgm of phencyclidine HC1 (sernylan). Four animals were also instrumented in the Group II study. One animal died from aortic rupture due to the Doppler flow transducer three weeks post-surgery. One animal was sacrificed 75 days post-operatively after suffering a cerebral infarction and early gangrene of the lower right leg arising from complications following cardiac catheterization and angiocardiographic studies. The last two animals remain alive and well at the present time. The telemetry units have operated well in all Group II animals. The unit implanted in one of the surviving animals in Group II continues to operate well eleven months post-operatively (Figure 3). The transmitter remains functional in the last animal but no useful physiologic signals have been obtained for the last two months. Data from these last three animals are included as Group II data in this study and were obtained both awake and following anesthesia.

Direct measurement of right and left sided pressures were made using Statham P23Gb gages from catheters inserted through the right and left femoral vessels using standard clinical catheterization techniques. 6F catheters were placed under fluoroscopic control in the right atrium for indicator dilution injection and infusion of sympathomimetic amines. Catheters were placed in the arterial system for indicator dilution sampling and measurement of arterial and left ventricular pressures. Reported levels of pressure represent average values before and after determination of indicator dilution cardiac outputs.

Cardiac output was measured in all animals by means of cardio-green indicator dilution methods with injection of 1.0 cc of dye into the right atrium and sampling in the aortic arch by an 8F fiber optic catheter system inserted by cutdown through the left femoral artery.[19] Pressure and flow responses of the central circulation were studied during constant infusions of standard sympathomimetic drugs into the right atrium using a Harvard syringe (Figures 4 and 5). Such studies were conducted 6 weeks post-surgery in Group I animals and 8 weeks post-surgery in Group II animals. Infusion of each drug concentration was maintained for a period of five minutes, hemodynamic measurements were made after three minutes of infusion during each period. Fifteen-minute recovery period was allowed between isoproterenol and norepinephrine infusions.

RESULTS

Figure 6 demonstrates the course and magnitude of heart rate and peak systolic right and left ventricular pressures for all Group II animals for a period from the day of operation to 150 days post-surgery. Figure 3 shows a detailed tracing obtained by telemetry in Chim-

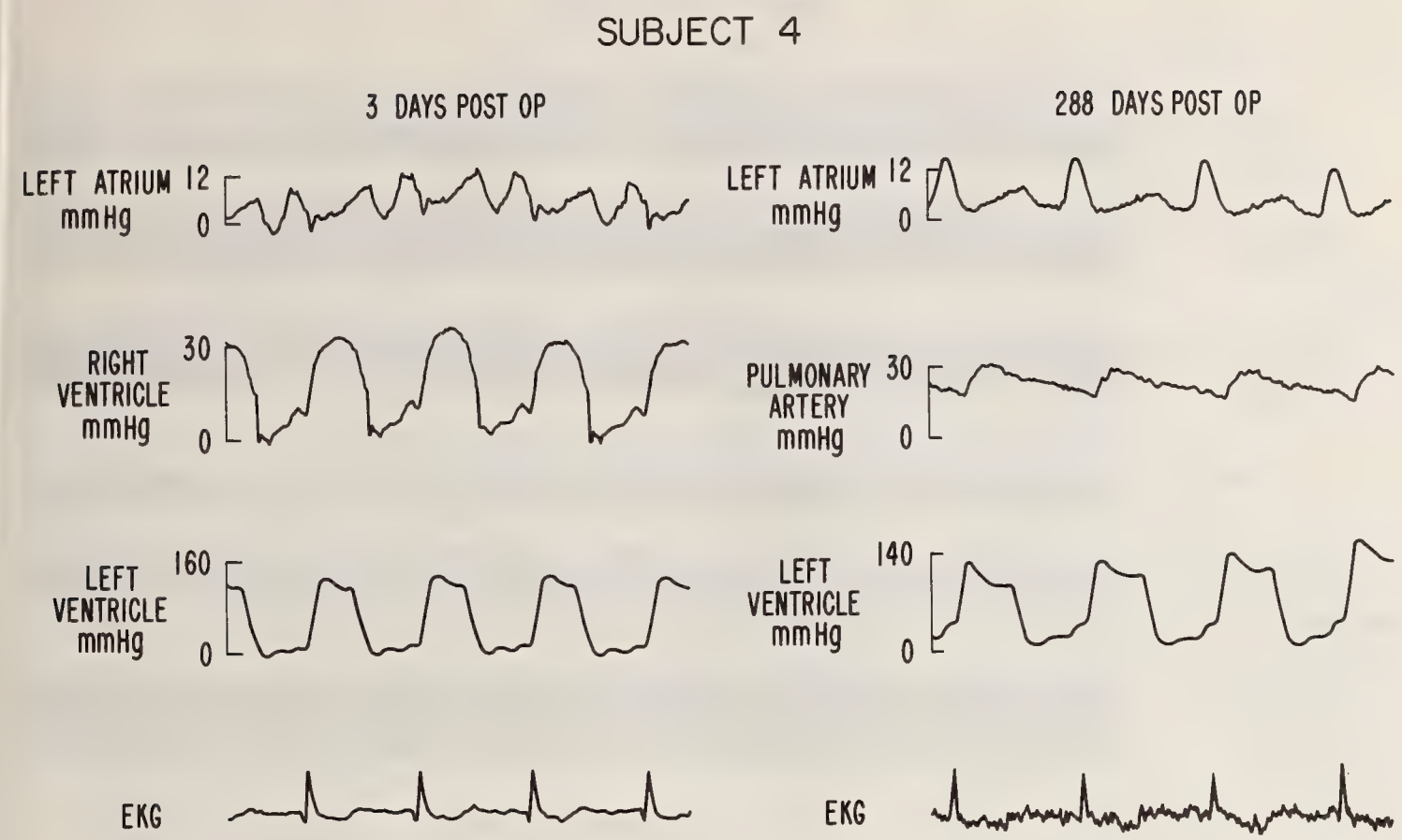


FIGURE 3.—Comparison of telemetry data taken 8 months apart.

panzee 3, 288 days after surgical implantation of the telemetry unit. This unit continues to remain functional. Analysis of Figure 6 demonstrates that both heart rate and pressure appear to be similar to human levels when such measurements are made in the unrestrained animal.

Figure 7 illustrates a typical tracing demonstrating the effects of phencyclidine (sernylan) anesthesia in these animals. Resting levels of heart rate and blood pressure were obtained 30 minutes prior to anesthesia, followed by squeezing of the animal which produced a marked increase in heart rate and blood pressure in order to deliver the intramuscular medication. By 20 minutes after injection, heart rate and blood pressure had returned to near baseline levels with little further change. Heart rate during anesthesia appeared slightly slower (5 to 10 beats/minute) and left ventricular systolic pressure slightly lower (10 to 20 mm Hg). Sinus arrythmia was accentuated in all animals. It was concluded from these observations made in three animals that sernylan did not augment heart rate or blood pressure response compared to the control or resting state.

Table I summarizes the response of seven animals to isoproterenol and norepinephrine infusion. All measurements were made during sernylan anesthesia. Flow increased slightly during isoproterenol infusion compared to resting values as did heart rate with little or a slight decrease in resulting stroke volume. Mean arterial pressure showed little change except at high infusion rates where there was a slight decline. Response to norepinephrine revealed no significant change in cardiac output and the presence of a significant bradycardia resulting in an increased stroke volume. Mean arterial pressure did not increase significantly except at higher infusion rates.

Figure 8 illustrates the use of these units to obtain heart rate and left ventricular pressure readings (chimp 4, Group II) over a 24 hour period three months after surgery. The unit was turned on and off remotely and telemetered recordings obtained on magnetic tape. A diur-

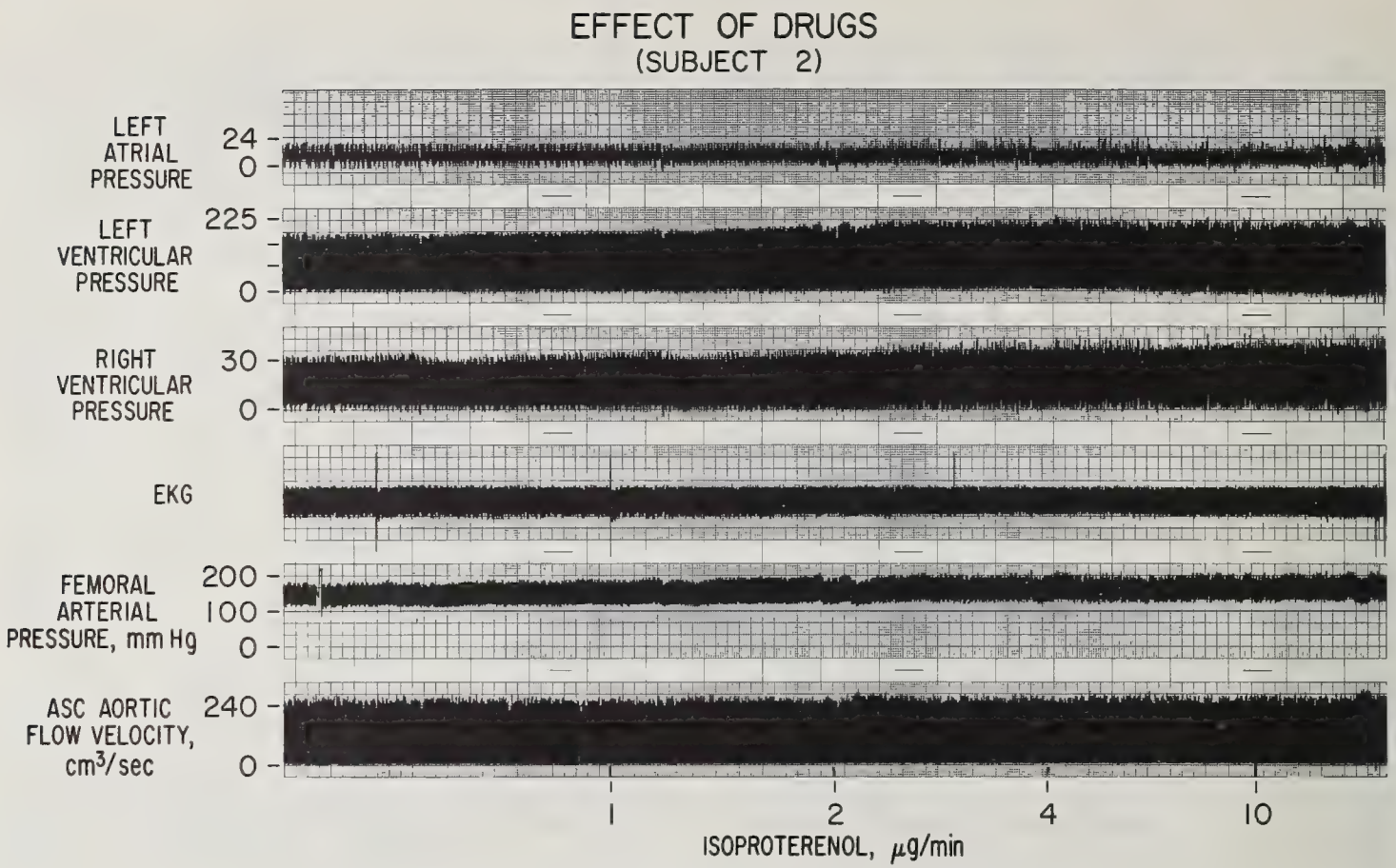


FIGURE 4.—Effects of infusion of isoproterenol in one animal.

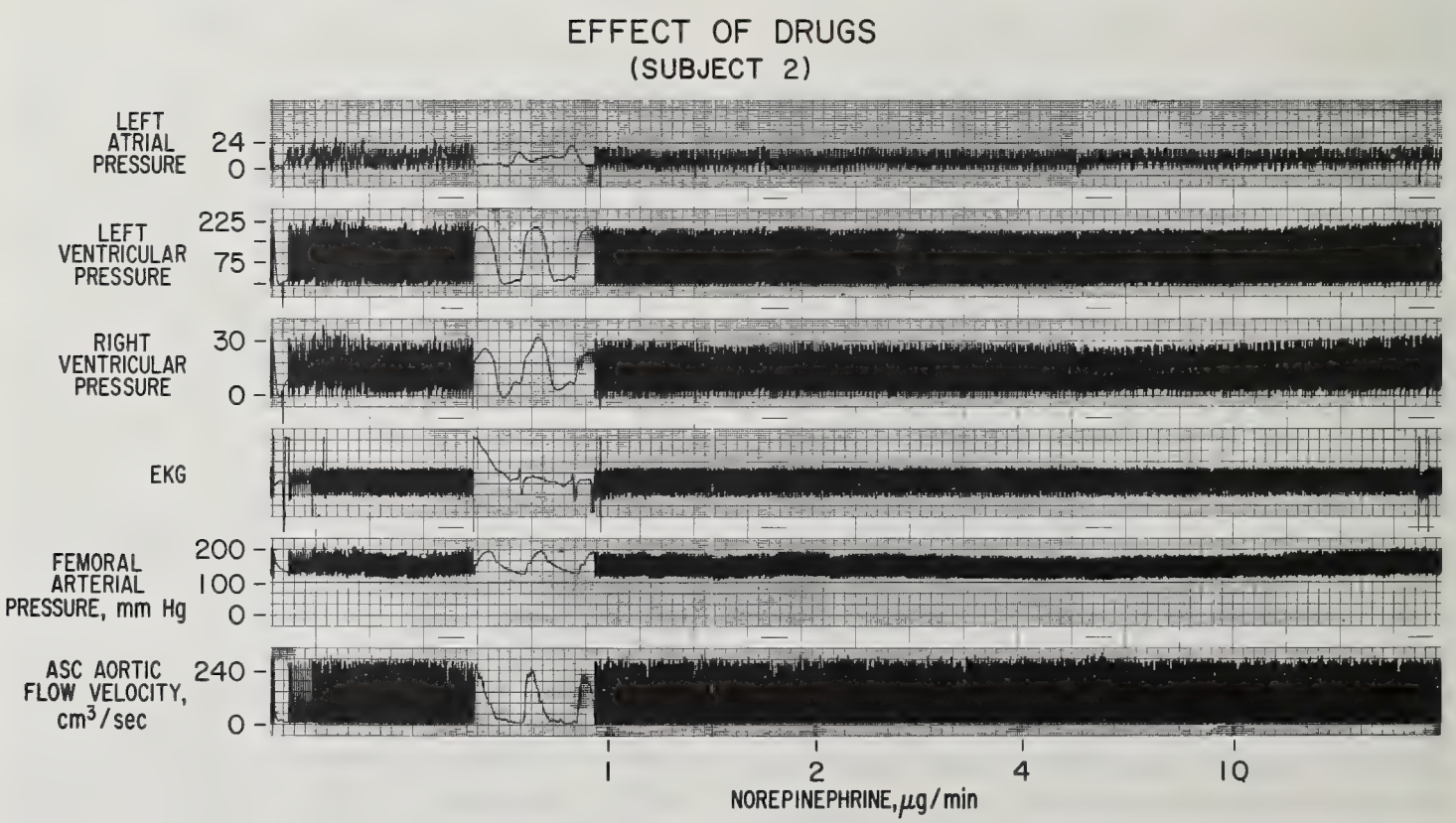


FIGURE 5.—Effects of infusion of norepinephrine in one animal.

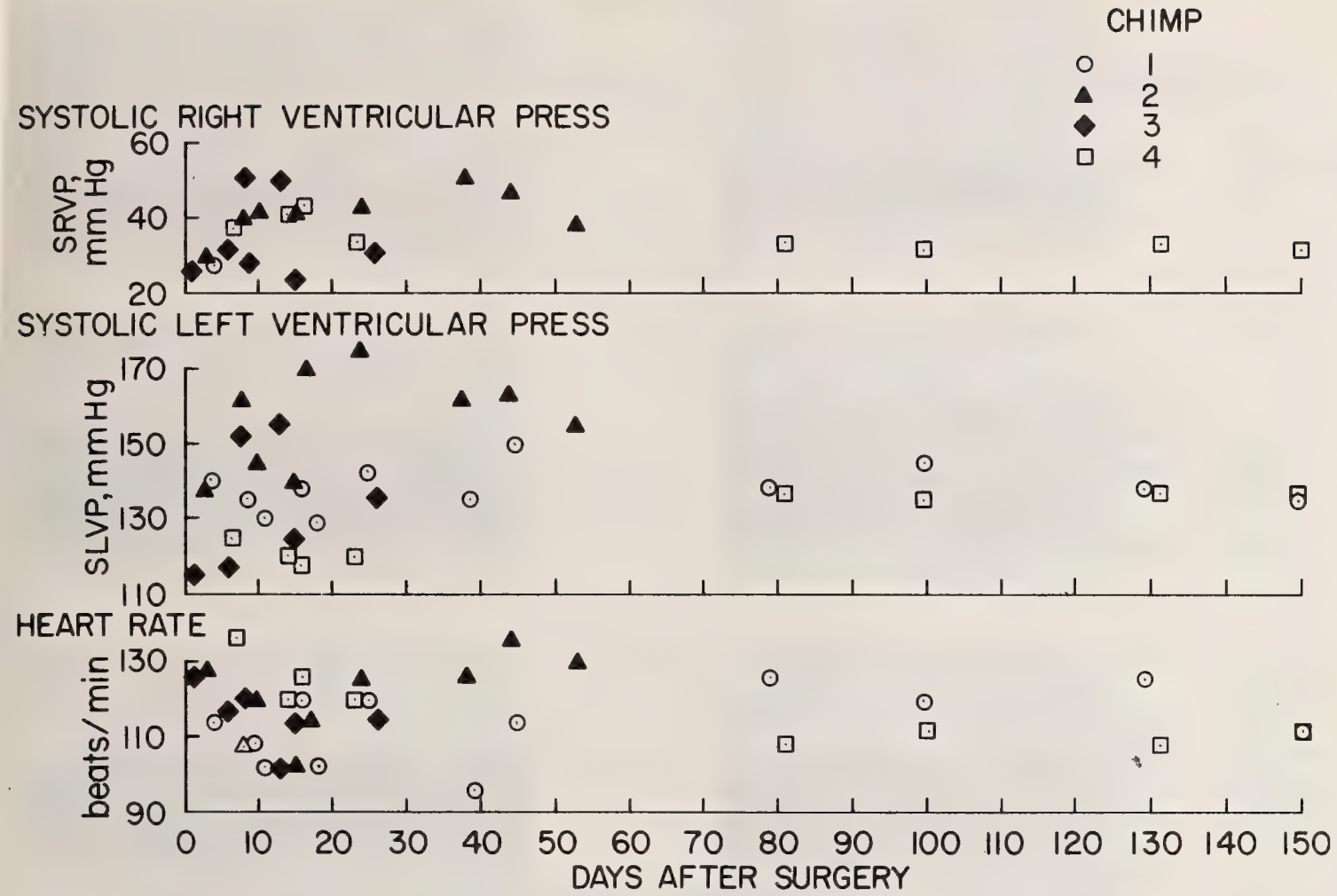


FIGURE 6.—Summary of pressure data for all animals in this study.

nal or circadian variation in pressure and heart rate are clearly demonstrated.

DISCUSSION

Previous hemodynamic studies in the chimpanzee have demonstrated the findings summarized in Table II. Such findings have demonstrated elevated heart rates and mean arterial pressures by human standards. Response to ganglionic blockade and tilt have lead Schoenfeld and co-workers to conclude that heart rate is predominantly under sympathetic control rather than parasympathetic control as it is in man.[9] The findings of this study support these conclusions. The use of implantable telemetry units has demonstrated markedly lower resting heart rate levels when compared to those previously reported in the literature. These findings agree with those previously reported by Weissler who found that spontaneous heart rate changes could occur during the recording of routine ECG tracings depending on the emotional arousal of the animal.[7] Marked sinus arrythmia was regularly found in this later study when heart rates decreased below 125 beats/min. These observations may help explain our findings during sernylan anesthesia where marked sinus arrythmia occurred. Heart rates are regularly below 125 beats/min with such anesthesia which also blocks emotional factors which may heavily influence observed findings in this animal.

The results of the study demonstrate that techniques for studying cardiovascular response in the chimpanzee are now available and can be used with reasonable success for studying varying stresses. These techniques allow for observations in an awake and completely unrestrained animal which is known to be highly uncooperative. These studies also show a labile highly reactive cardiovascular state. Although many

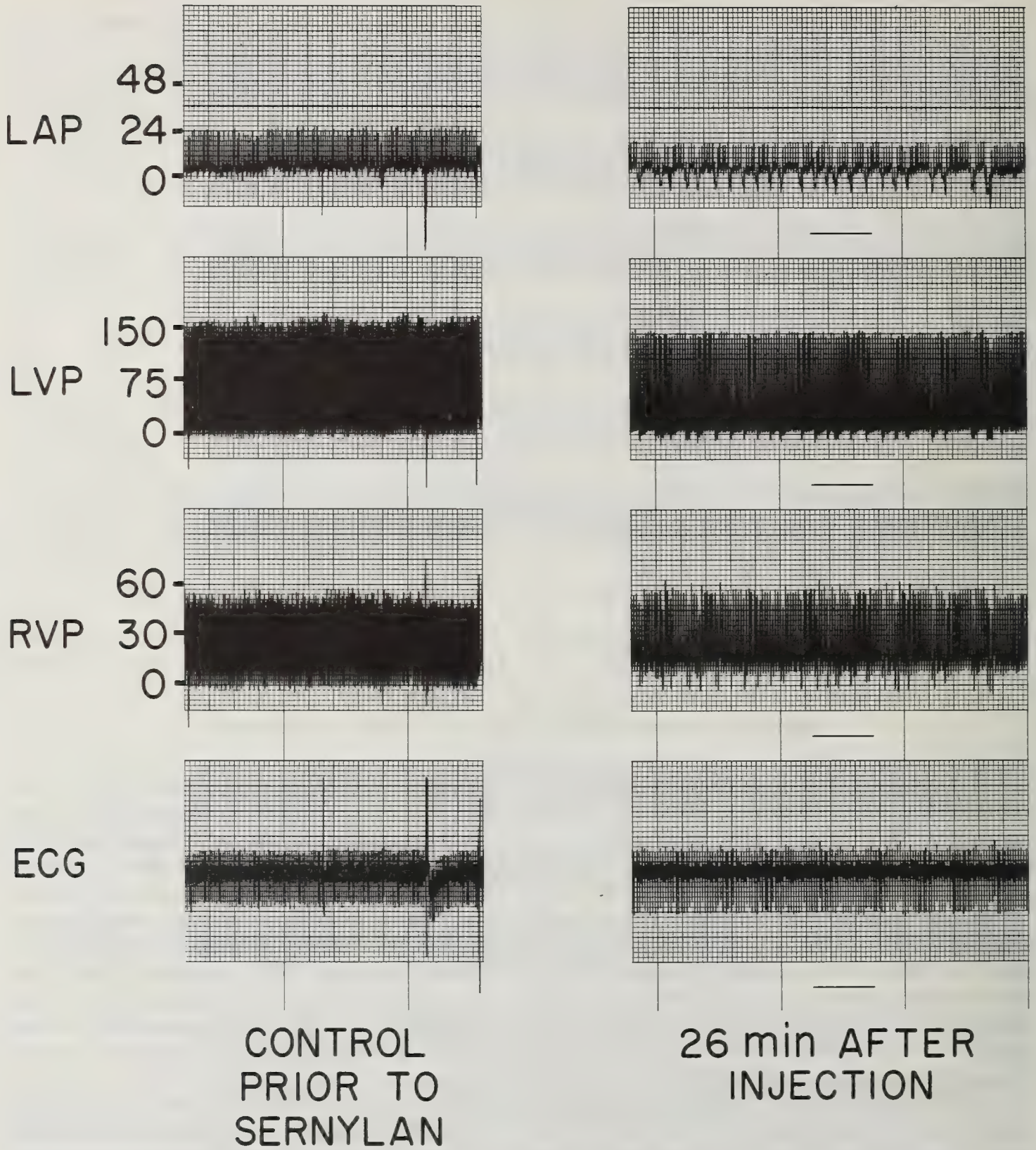


FIGURE 7.—Effects of intramuscular injection of Sernylan.

similarities are present between the cardiovascular response of the chimpanzee and man, areas of apparent difference are becoming apparent as in-depth studies are being conducted. Although additional studies must be conducted before definitive statements can be made, it appears that previously reported findings of elevated heart rate and blood pressure may be

TABLE I.—*Response to Isoproterenol and Norepinephrine Infusion.*

RESPONSE TO ISOPROTERENOL AND NOREPINEPHRINE INFUSION

CONDITION	DOSE μg/min	MAP mm Hg	HR beats/min	CO l/min	SV cc/beat
CONTROL	–	136 ± 3*	107 ± 4	3.51 ± 0.20	33 ± 2
ISOPROTERENOL	0.5	141 ± 4	114 ± 3	3.60 ± 0.35	31 ± 2
	1.0	144 ± 6	128 ± 14	3.60 ± 0.35	28 ± 2
	2.4	151 ± 8	128 ± 12	3.60 ± 0.33	28 ± 2
NOREPINEPHRINE	1.0	135 ± 2	102 ± 5	3.51 ± 0.30	35 ± 3
	2.0	140 ± 9	111 ± 7	3.52 ± 0.41	35 ± 2
	4.0	146 ± 3	99 ± 7	3.50 ± 0.34	35 ± 4

* REPRESENTS ONE STANDARD DEVIATION OF THE MEAN
MAP = MEAN ARTERIAL PRESSURE
HR = HEART RATE
CO = CARDIAC OUTPUT
SV = STROKE VOLUME

more apparent than real and due more to emotional factors than to basic differences in species. It does appear that different control mechanism may be used by the chimpanzee and further work is required to quantify such differences. At present, enough similarities exist to support use of the chimpanzee as a model for man. Such a model would allow for testing of noninvasive instrumentation techniques and for the use of implanted electronic packages such as the radiotelemetry unit used in the present study. This testing will allow for subsequent future recommendations for use of such advanced technology in man either directly or as part of other systems such as an implanted artificial heart.

SUMMARY

Cardiovascular measurements have been obtained in chimpanzees using completely implantable telemetry units.

Recordings of intravascular pressures and heart rates obtained by such methods were lower than previously reported values obtained from restrained and/or anesthetized animals.

Preliminary investigations demonstrate that different cardiovascular control mechanisms may be used by the chimpanzee compared with man, but further work is required to quantify such differences.

Results with the implantable systems are encouraging and allow for the recommendation of the use of such devices in man at a future date,

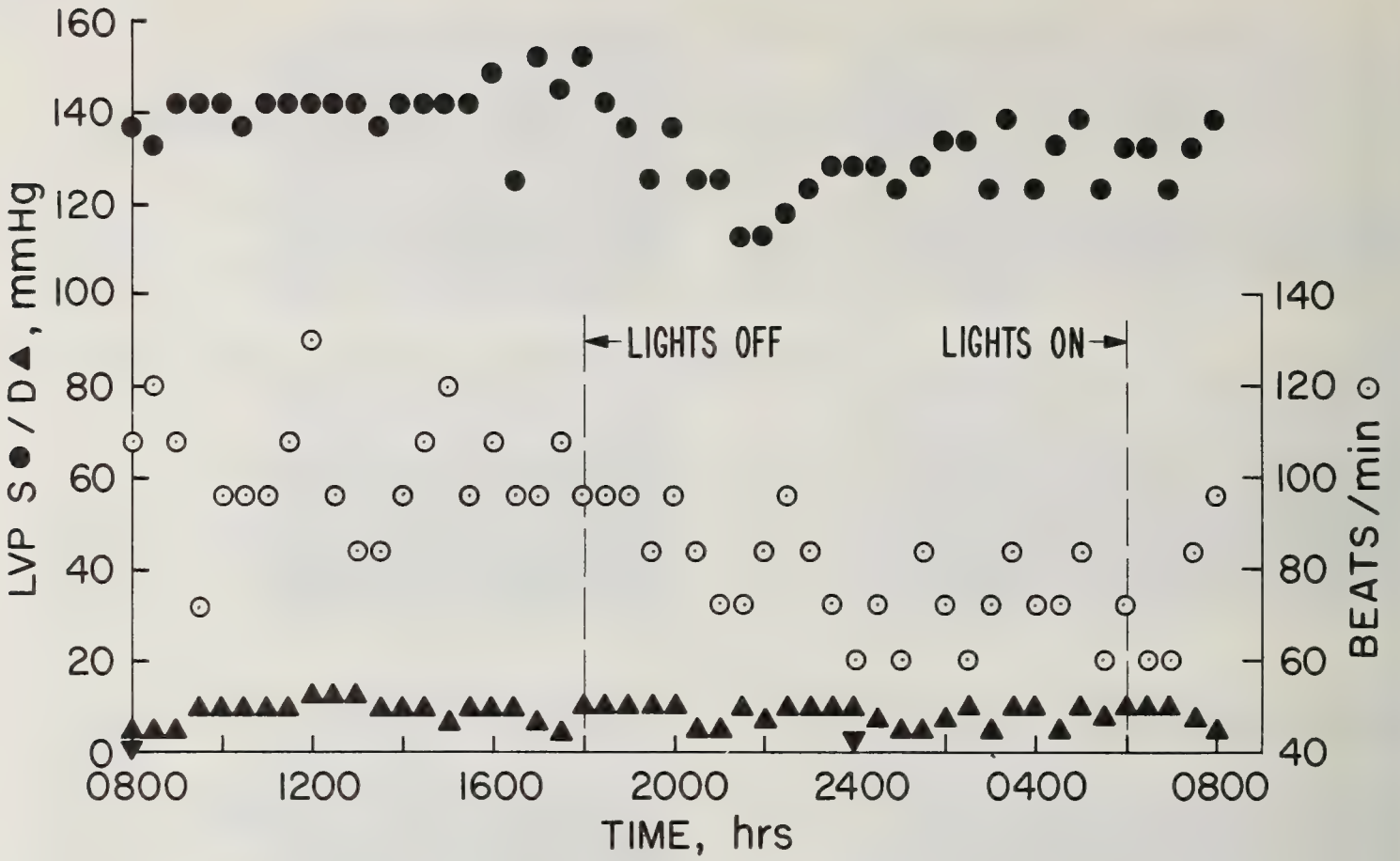


FIGURE 8.—Summary of 24-hour data in subject 4.

either alone, or as part of devices implanted in or about the cardiovascular system.

REFERENCES

1. KRATOCHVIL, C. H. Antropoid apes in experimental medicine: A review. Ann. N. Y. Acad. Sci. 162:301–310, 1969.
2. BUXTON, D. F., REITE, M. L., and JACKSON, W. J. Some research techniques applicable to study of the chimpanzee nervous system. In: *Primates in Medicine*. H. H. Reynolds, Ed. S. Karger, Switzerland, 1969, pp. 52–61.
3. MASON, W. A. Chimpanzee social behavior. In: *The Chimpanzee*. Vol. 2. G. H. Bourne, Ed. S. Karger, Switzerland, 1970, pp. 265–288.
4. MCCLURE, H. M., and GUILLOUD, N. B. Comparative pathology of the chimpanzee. In: *The Chimpanzee*. Vol. 4. G. H. Bourne, Ed. S. Karger, Switzerland, 1971, pp. 104–244.
5. HENRY, J. P., and MOSELEY, J. B. Results of the Project Mercury Ballistic and Orbital Chimpanzee Flights. National Aeronautics and Space Administration Technical Report SP-39, 1963.
6. HARDY, J. D., CHAVEZ, C. M., KURRUS, F. D., NEELY, W. A., ERASLAN, S., TURNER, M. D., FABIAN, L. W., and LABECKI, T. D. Heart transplantation in man. Developmental studies and report of a case. J.A.M.A. 188:1132–1140, 1964.
7. WEISSLER, A. M., FINEG, J., and WARREN, J. V. The Electrocardiogram of the Young Chimpanzee. Air Force Technical Report, 6571st Aeromed. Res. Lab., Holloman, AFB, N. Mex., MDC-TDR 61-29, 1961, p. 1–27.
8. WEISSLER, A. M., SCHOENFELD, C. D., CARTER, V. L., COHEN, S., STILSON, A. E. JR., and WARREN, S. V. The Effects of Psychomotor Arousal, Hemorrhage and the Upright Posture on the Circulation of the Young Chimpanzee. Air Force Technical Report, 6571st Aeromed. Res. Lab., Holloman AFB, N. Mex., ARL-TDR 63-28, 1963, pp. 1–30.
9. SCHOENFELD, C. D., WEISSLER, A. M., CARTER, V. L., and WARREN, J. V. The Effects of the Ganglionic and Adrenergic Blockade on the Circulation of the Young Chimpanzee. Air Force Technical Report, 6571st Aeromed. Res. Lab., Holloman AFB, N. Mex., ARL-TDR 66-7, 1966, pp. 1–23.
10. DUCKER, T. B., SIMMONS, R. L., and ANDERSON, R. W. Increased intracranial pressure and pulmonary edema: 3. The effects of increased intracranial pressure on the cardiovascular hemodynamics of chimpanzees. J. Neurosurg. 29:475–483, 1968.

TABLE II.—*Summary of Cardiovascular Data on the Chimpanzee From the Literature.*

	NUMBER OF ANIMALS	HEART RATE BEATS/min	CARDIAC OUTPUT liters/min	STROKE VOLUME cc/beat	MEAN ART. PRES mmHg
RESTING					
WEISSLER[12]	25	148 ± 24	3.07 ± 0.36	21 ± 4	134 ± 24
SCHOENFELD[18]	6	148 ± 10.7	3.72 ± 0.96	25 ± 5.9	136 ± 24
HAMLIN[19]	9	—	3.24 ± 0.55	—	—
PRESENT STUDY	7	107 ± 4	3.51 ± 0.20	33 ± 2	136 ± 3
BETA BLOCKADE[18]					
BEFORE	6	148 ± 10.7	3.72 ± 0.96	25 ± 5.9	136 ± 24
AFTER		116 ± 4.6*	2.79 ± 0.58	24 ± 3.6	130 ± 28
ALPHA BLOCKADE (PHENOXYBENZAMINE)					
BEFORE	6	152 ± 15	3.52 ± 0.57	23 ± 4.3	144 ± 20
AFTER		176 ± 19	3.42 ± 1.25	19 ± 5.7	106 ± 29*
GANGLIONIC BLOCKADE[18]					
BEFORE	6	147 ± 8.2	3.94 ± 0.61	27 ± 6.5	146 ± 27
AFTER		114 ± 16.6*	2.56 ± 0.85*	22 ± 7.6*	98 ± 24*
90° TILT[12]					
BEFORE	13	145	3.17	23	128
AFTER		166	2.66	17	125
90° TILT WITH ALPHA BLOCKADE[18]					
BEFORE WITH ALPHA BLOCKADE	8	176 ± 17	3.54 ± 1.2	20 ± 6	104 ± 22
AFTER WITH ALPHA BLOCKADE		180 ± 15	2.41 ± 0.83*	14 ± 4*	86 ± 13*

*SIGNIFICANT DIFFERENCE, $p < 0.01$

11. FRANKLIN, D. L., and VAN CITTERS, R. L. Blood flow in mesenteric artery of chimpanzee, monitored via radiotelemetry. Aero. Med. 38:926–931, 1967.
12. PARRATT, J. R. The effects of histamine on the myocardial circulation. Eur. J. Pharm. 6:203–208, 1969.
13. SIMMONS, R. L., DUCKER, T. B., and ANDERSON, R. W. Pathogenesis of pulmonary edema following head trauma: An experimental study. J. Trauma 8:800–811, 1968.
14. FRYER, T. B., SANDLER, H., and DATNOW, B. A multichannel implantable telemetry system. Med. Res. Eng. 8:9–15, 1969.
15. SANDLER, H., STONE, H. L., and FRYER, T. B. The use of implantable telemetry systems for study of cardiovascular phenomena. Circ. Res. (in press) 1972.
16. SANDLER, H., FRYER, T. B., WESTBROOK, R. M., and KONIGSBERG, E. Miniature implantable accelerometers. In: *Implanted Cardiovascular Instrumentation.* E. P. McCutcheon, Ed. Academic Press, Inc. N.Y. (in press) 1972.
17. LINEBARGER, R. N., LIM, R. S., SANDLER, H., and FRYER, T. B. Real time computer measurement of heart dimensions from implanted accelerometers. Proc. Conf. Eng. Med. Biol. 9:31.2, 1967.
18. STONE, H. L., SANDLER, H., FRYER, T. B. Cardiovascular function in the chimpanzee studied by chronic instrumentation techniques. In: *The Chimpanzee.* Vol. V. G. Bourne, Ed. S. Karger, Switzerland (in press) 1972.

19. HUGENHOLTZ, P. G., WAGNER, H. R., and ELLISON, R. C. Application of fiber optic dye dilution techniques to the assessment of myocardial function. Amer. J. Cardiol. 24:79-94, 1969.

DISCUSSION

CHAIRMAN HAWTHORNE: Thank you Dr. Sandler. Do you keep these chimps in the squeeze cages all of the time?

DR. SANDLER: Yes, we've not been turning them loose in the runs.

CHAIRMAN: Would the pressures change more if you let them run around for awhile?

DR. SANDLER: This is what we would like to do. But, initially we had a lot of trouble getting the units working. Now we have a reliable implantable telemetry unit which can operate for periods in excess of one year. The range of transmission of the telemetry units is 50 to 100 feet which limits us to a certain extent because if we turn them loose in a compound, there would be areas in the compound where we would have trouble picking up a signal. This would require the use of a transponder or a retransmitter. In other words, the animal would have to probably wear some little jacket or unit and carry something about the size of a cigarette lighter in the unit to pick up the weaker implanted signal and then retransmit for as far as we need. We have not done that particular part of it, that's why we've stuck with the squeeze cage.

CHAIRMAN: Well I'm glad to see that the chimp is not habitually hypertensive.

SOMANI, PITAMBAR, Abbott Laboratories, North Chicago, Ill.: I was stuck by your findings that isoproterenal did not seem to produce as much effect as we expect it to in other animals. Were you anesthetizing these chimpanzees? And the response was unexpected because in the dog and other animals during the anesthetizing stage we get an increase in cardiac output, increase in left ventricle pressure, increase in mean arterial pressure and it increases peripheral resistance. In looking at your slide, there was only an increase in peripheral resistance and mean arterial pressure.

DR. SANDLER: When one studies awake and anesthetized dogs, one can obtain the types of response you mentioned here. I will agree with you that you could predict that isoproterenal would do the things that you said. But it did not occur in this animal and it is of interest to think that the chimp is not controlled or driven in the manner in which man or the dog is. I think, we find the dog could be characterized in his control system as being primarily parasympathetic, man is mixed, and that most primates, and I think the chimp is a classic example of this, would be primarily sympathetically driven. We're seeing some evidence of this primary sympathetic drive in the response of isoproterenal, but the small number of animals we have here is not enough to make any statement as strong as that yet. But our findings make us think that we should do more work in that area.

DR. SOMANI: Which beta blocking drug are you using?

DR. SANDLER: Propranolol.

DR. SOMANI: Do you have any idea about coronary occlusions in the chimpanzee?

DR. SANDLER: No. We have not done this nor is there anything, unless we've missed it, in the literature which would make us believe this. We're interested about coronary occlusions in the chimpanzee especially in evaluation of how good a cardiac model the chimpanzee is for man. We found that, first of all, the control system is not a direct analog. The second, that when we went to do angiocardiograph studies we found the chimp has a completely horizontal heart. When the chimp is laying on his back, the heart is just like a gun barrel, where man's heart position is different. We're also finding slight differences in the dynamic geometry with the contraction pattern. But, these are very early findings.

J. T. REEVES, University of Kentucky, Lexington, Ky.: In your best animals, how do you feel that you are approaching normal in regards to respiration, arterial blood gases, and other vital measurements?

DR. SANDLER: We've only had four aninals live beyond thirty-eight weeks, but the one animal that has survived from our first studies, who is now two years exactly post-op, has gained thirty kilo in weight. He started out being about forty pounds and now he's almost 100 pounds, besides being difficult to handle. I think that gives us one piece of information,

that this unit has not hurt this animal. We've done periodic blood studies in terms of white counts, hemoglobin and the surviving animals have thrived. There has been no evidence of any ill effect from the implantation.

G. F. GESTRING, Newark Beth Israel Medical Center, Newark, New Jersey: What do you mean by zero estimation in a clinical fashion? And what about the ST changes?

DR. SANDLER: In terms of zero, we have used the reference point that is classically used which means the level of right atrial distance in the chest. We estimate where the level of the mid right atrium is and zero our pressure gauges when we use the standard clinical cardiac catherization technique. We have not made a study of ST changes. We find, however, after surgery, that there are significant ST changes, but you know we have incised both the right and the left ventricles in order to insert the pressure gauges through the wall of the left ventricle. However, the animal recovers back to a nomal position. We have not used any standard placing of EKG leads from the animals. At times we've experimented with this but we cannot make any real comments about where and what the significance of the EKG changes are at this time.

CHAIRMAN: I might say that almost everybody that uses this method of measuring pressure calibrates it from a comparative pressure, from say a Stathum transducer and a tube in the ventricle or chamber.

CHAPTER VII

PHYSIOLOGY

Comparative Electrocardiography

Physiologic and Pharmacologic Response of Chronically Denervated Feline Hearts

"Pacing" Left Ventricular Function Curves in Conscious Dogs

Electrocardiogram, Myocardial Action Potential and Cardiac Excitability in Laboratory Animals

An Animal Model of Isorhythmic Atrioventricular Dissociation

Pulmonary Function Testing of Unanesthetized Beagle Dogs

Changes in Left Ventricular Hemodynamics and Dimensions During Rejection of the Orthotopically Transplanted Canine Heart

COMPARATIVE ELECTROCARDIOGRAPHY

Robert L. Hamlin and C. Roger Smith*

Clinical and experimental observations indicate that the electrocardiogram has serious limitations in studying abnormalities of the heart in certain species (bovine, porcine, equine) in which inordinate degrees of cancellation occur during ventricular depolarization. In these species with a more complete penetration of Purkinje fibers throughout both ventricular free-walls, even massive ventricular hypertrophy or dilatation may produce no or very minimal alterations in body surface potentials; while production of massive zones of ischemia of the left ventricular free-wall merely alters QRS vectors to appear like normal vectors in mammals which do not have such complete penetration of Purkinje fibers.

If, in the pathogenesis of disease, alterations in ventricular activation are important, then animals with complete penetration of Purkinje fibers throughout both ventricular free-walls should not be used as models. For this reason, no matter how severe the heart lesion, ventricular activation may be changed only minimally. On the other hand, these species may serve as useful models to study the effects of lesions independent from changes in ventricular activation.

Larger species (e.g., equidae) are of interest because of their propensity for developing atrial arrhythmias—in particular, atrial fibrillation—because of the absolute greatness of their atrial mass; while smaller species (e.g., small dogs) are of interest because they may have most severe atrial disease without developing atrial fibrillation.

Another interesting aspect of comparative electrocardiography lies with phenomena considered normal in certain mammals (e.g., second-degree A–V block and P-mitrale in equidae, pronounced sinus arrhythmia in dogs, wandering atrial pacemakers in dogs and horses) but considered to be abnormal in man.

Differences exist in postures within the thorax of hearts of quadrupeds compared with hearts of primates; emphasizing that, even though cardiac activation may be identical in certain quadrupeds and primates, body surface potentials of one may be grossly different from surface potentials of the other.

INTRODUCTION

To make optimal use of the electrocardiograph for monitoring electrophysiologic properties of the heart, the investigator should be familiar with the normal electrocardiogram of the species used in the investigation. Too often an investigator will interpret an electrocardiogram obtained from one species using criteria established for another.

If, for example, one interprets the electrocardiogram from a normal horse, dog, cow, or sea lion, using criteria established as either normal for man or as indicative of certain abnormalities in man, one will claim the horse has left atrial enlargement because of the P-mitrale,[1,2] the dog has left ventricular hypertrophy because of the ST–T contour,[3] the cow has either right bundle branch block or right ventricular hypertrophy because of orientation of mean and terminal vectors of QRS,[4,5] and the sea lion has atrial injury because of the PQ segment deviation.[6]

DISCUSSION

Variations in the form of the electrocardiogram among the various species might arise for two reasons: either the cardiac activation process varies, or the position of the heart within the torso varies.

We would like to present examples of these two determinants and to classify[7] many domestic mammals according to the form of QRS.

Components of QRS are produced by uncancelled boundaries between electrically active and resting ventricular myocardium. Thus, the time-course of ventricular depolarization determines the form of QRS in any lead. That time course is determined by the distribution of Purkinje tissue within the ventricular mass. Thus, we proposed that differences in form of QRS reflect differences in the distribution of the Purkinje fibers in ventricles of the various individuals of each category of mammal.

* Department of Veterinary Physiology & Pharmacology, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210.

If one examines a sagittal cross-section of a typical mammalian heart (Figure 1), boundaries between depolarized (stimulated) myocardium and resting (unstimulated) myocardium may be established at any instant during QRS. This has been accomplished [8–10] by impaling the heart with numerous multipolar electrodes from which characteristic electrical potentials are recorded when the wave of depolarization reaches them.

In animals of category I (carnivores [3,8,9,11] and primates,[12]) ventricular depolarization proceeds with 4 general fronts of activity. During the initial portion of QRS (boundaries labeled 1), the apical-third of the interventricular septum is excited from each ventricle toward the middle

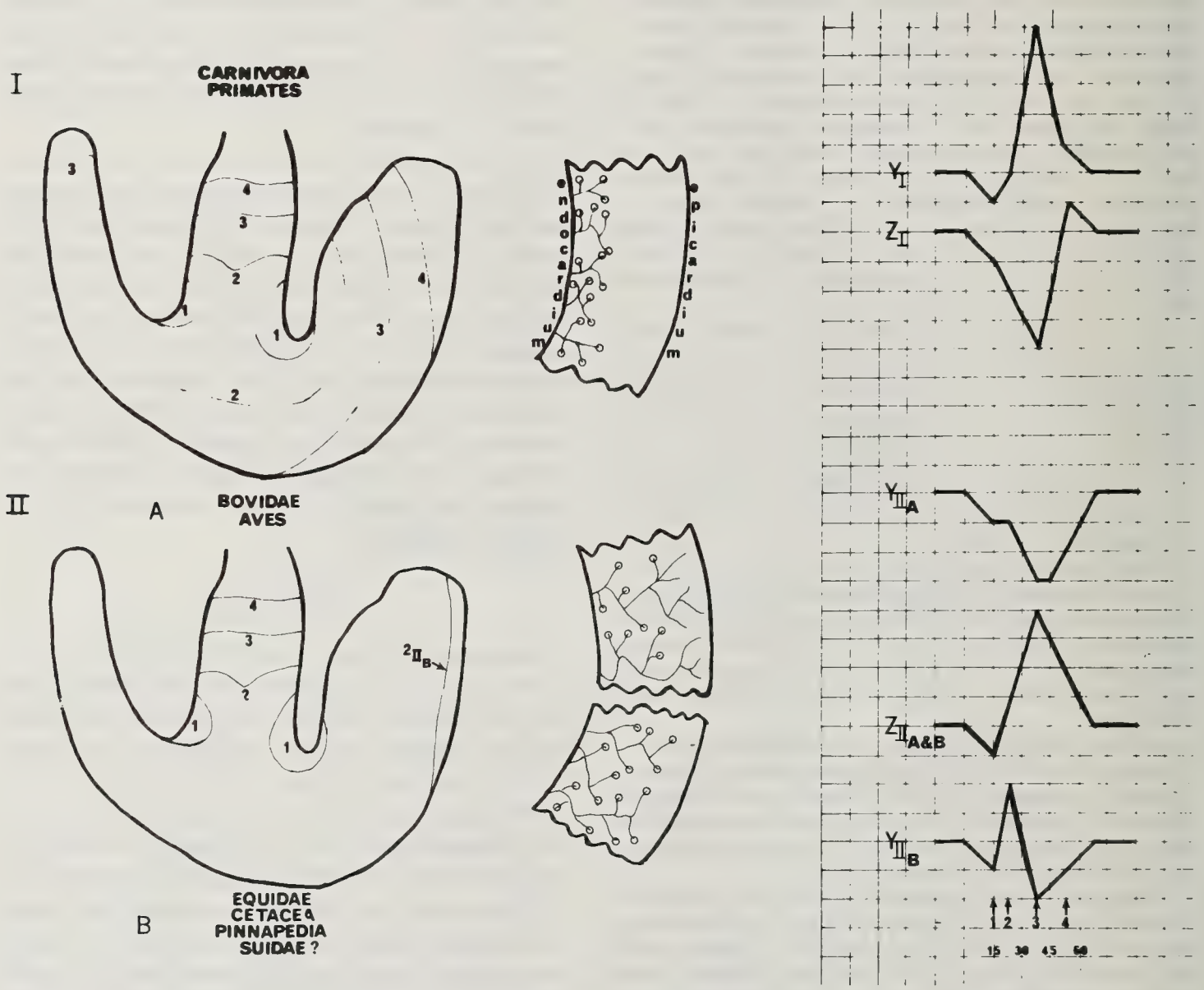


FIGURE 1.—Schematic drawings of the sagittal cross-section of the hearts of animals in two major categories. Boundaries between depolarized and resting myocardium at 4 instants during ventricular deplorization are labeled 1 through 4. To the right of each heart is a schematic drawing of the left ventricular free-wall showing the degree of penetration of Purkinje fibers. The bottom free-wall is divided with the top half representing Purkinje fibers penetrating the entire endocardial to epicardial distance, while the bottom half shows penetration only to the subepicardium, Y and Z axis electrocardiograms appear to the right, with arrows and time in msec. after onset of QRS corresponding to the boundaries identified in the hearts to the left. Y axis refers to the craniocaudal, and Z axis to the dorso-ventral axes of the torso.

of the septum; but the contribution of the left ventricle is greater than that for the right. This activity generates Q-waves in leads Y and Z, since the exploring electrode of each lead "sees" a boundary of negativity "leaving" it.

Next, as the activation process is expedited to the end-points of Purkinje fibers penetrating from ¼ to ⅓ of the endocardial to epicardial distance into both ventricular free-walls, the boundary of activity (labeled 2) proceeds in a general endocardial to epicardial direction through the entire ventricular mass, as well as ascending the interventricular septum in an apico-basilar direction. This activity produces little electrical disturbance in the Y axis lead, but a large negative deflection in the Z axis lead since it is "seen" as a large negative boundary "leaving" that electrode.

During the instant when the deflection of greatest magnitude is inscribed on the electrocardiogram, a large boundary of activity (labeled 3) traverses the thick-walled left ventricle in a subepicardial to epicardial direction, as well as continuing in an apico-basilar direction up the middle to basilar third of the interventricular septum. This activity produces a large R-wave in the Y axis lead and a continuing negative deflection in the Z axis lead.

Lastly, the base of the interventricular septum and bases of both ventricles are activated in an apico-basilar direction (labeled boundary 4). This activity is responsible for generating a R-wave in the Z axis lead; and, depending upon its spatial orientation to the Y axis lead, either a S-wave, a r-prime, or merely a "sloppy" descending limb of the R-wave.

Animals classified in this group all have QRS complexes in the Z axis lead of the Qr contour.

Animals in category II have a Z axis QRS of the qR pattern. Animals in category IIA have a Y axis QRS of the QS or rarely qrS; while animals in category IIB have a Y axis QRS of usually a qRS.

In animals of category IIA (ruminants [8,13,14] and birds,[15]) ventricular activation proceeds with only 2 general fronts of depolarization. During the initial portion of QRS, the apical third of the interventricular septum is activated in precisely the same manner as that in animals of category I, and q-waves are generated in both Y and Z axis electrocardiograms.

During the remainder of QRS, a boundary ascends the interventricular septum in an apico-basilar direction. This activity generates a R-wave in the Z axis lead and usually a S-wave in the Y axis lead.

Both ventricular free-walls are excited with a burst of activity in species of this category because of the complete penetration of the Purkinje system from endocardium to epicardium.[16] Because of anastomoses between first or second generation branches of both main bundles in the apex, little or no alteration in ventricular activation occurs even when one sections either main bundle.[4] In birds, Davis [17] describes penetration of Purkinje fibers from epicardium to endocardium following major intramural coronary arteries into the transmural depths. However, because of the complete transmural penetration, even though from epicardium to endocardium, free-wall activation in birds simulates that in ruminants.

In animals of category IIB (equidae,[18] cetaceae,[19] pinnapeds,[6] possibly swine,[20]) ventricular activation proceeds with the same two general fronts as in species of category IIA; but, after the initial septal activation and at the time the apical to middle third of the septum is being activated, an intermediate boundary excites the epicardum of the left ventricular free-wall. This activity generates a R-wave in the Y axis electrocardiogram, usually of low magnitude and short duration.

The genesis of this activity is equivocal, and we propose three possibilities. If the thin epicardial layer of the left ventricular free-wall were void of Purkinje tissue;[21,22] or, if Purkinje tissue were present but conducted at a slower velocity because of it being of small diameter,[23] then a front of sufficient magnitude to contribute to genesis of QRS might be produced by activity of this zone.

The alternative explanation is that even though the Purkinje fibers do penetrate the entire endocardial to epicardial transmural dimension, because they conduct at a velocity of from 2 to 5 M./second,[23] time skewing in activation between endocardial and epicardial layers might produce a boundary between these layers.

In computer-simulated plots of ventricular activation in animals[24] with Purkinje fibers penetrating as we propose in animals of this category, enough asynchrony of activation between endocardium and epicardium exists to generate such a front.

Indeed, this might account for the apparent lability in contour of QRS in the same horse from beat to beat or among different individual cattle. In the horse (Figure 2) QRS in the Y axis electrocardiogram may vary from a QS to a qrS pattern. Possibly QRS in the former pattern arises from accelerated conduction over Purkinje fibers permitting complete cancellation of fronts during free-wall activity; while, in the latter case, Purkinje fiber conduction may be slowed and sufficient asynchrony results to generate a R-wave.

With respect to the electrocardiogram being "blind" to ventricular disease, animals in group II propose a particular problem. Since most of the ventricular mass in individuals of those

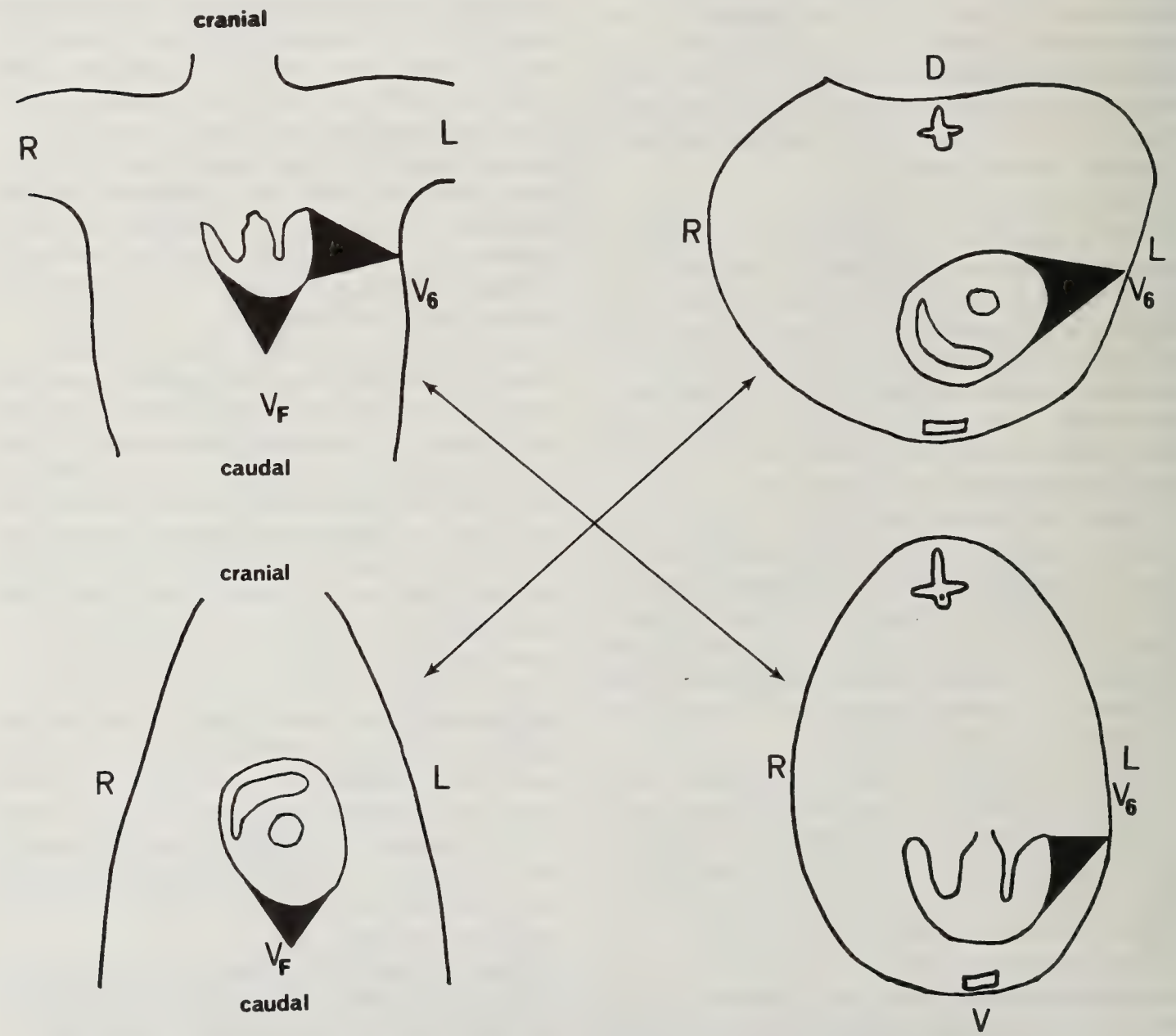


FIGURE 2.—Schematic drawings of cross-sections through the human and canine torso. R = right side, L = left side, D = dorsal side, and V = ventral side. Black cones are constructed from points on the torso where unipolarly-recording electrodes are placed to the face of the ventricle they "see." Criss-crossing arrows connect cross-sections of the torsos which contain cross-sections of the heart that appear analogous.

species is activated with a burst of cancelling activity, it is electrocardiographically silent. Therefore, disease—including gross enlargement—of either ventricle is obscured. One interesting feature of the these species,[25] however, is that the left ventricular epicardium may be "dePurkinjenated" by producing ischemia of that region. Thus, the goat may be converted into a dog or man by merely removing the additional Purkinje tissue with which he was endowed by his species superiority. We may speak of this as superiority because his complete penetration of Purkinje tissue expedites the activating stimulus to all regions of the heart without need for much myocardial to myocardial activation; plus the fact the goat cannot develop bundle branch block because of the previously mentioned anastomoses between the main bundles.

As mentioned previously, differences in the form of body surface potentials between individuals of the same species or different species may arise not only from differences in cardiac activation processes, but also from differences in the spatial orientation of the heart within the torso. Thus, the ventricular activation process of two animals may be identical, but QRS from identical leads in each animal may vary markedly.

We may presume that the ventricular activation process of man and dog are similar; yet QRS's from leads I, II and III in man are different in configuration than from the same leads in the dog. Previously, we have heard of the dog heart as being similar to man's only electrically vertical; since, if QRS's in leads I, II and III in man appeared as they do in a dog, normally, the man would be considered as having an electrically vertical heart.

This is deceptive nomenclature, however, since it infers that when the human heart lies vertically within its torso, radii drawn to the epicardial perimeter of the heart from lead aV_F would subtend a portion of that heart which would be similar to that subtended in a like manner from lead aV_F in the dog. As shown (Figure 3), this is untrue. Indeed, lead aV_F of man "looks at" the left ventricular apex; while the same lead, in the dog, "looks at" the left ventricular free-wall. What is termed the anterior (or truly the ventral) surface of the human heart is, for the dog, the sinistrad surface. A frontal (or ventral) cross-section cut through the human heart parallels, more closely in the dog, a horizontal or coronal section. A frontal (ventral) cross-section cut through the dog heart parallels, more closely in man, a horizontal section.

Another factor of importance[26] in attempting to draw analogies between electrocardiograms for man and dog is the dependency, in the dog, on the position of the thoracic limbs with respect to the torso. Anatomical peculiarities to quadrupeds dictate that both thoracic limbs must be placed so that the humeri arise at a near right angle with the long axis of the torso. This is particularly important for the left thoracic limb; for it is pulled caudad, the form of QRS in av_L may change from the normal Qr to an atypical qR.

It should be considered, also, that lead I in man, which measures the potential difference between two thoracic limbs which attach to the torso at least near the geometrical X axis of the torso, has no simple analogue in the dog. For both of dog's thoracic limbs attach to the torso far craniad to the heart. Thus unipolar electrocardiograms from both thoracic limbs appear nearly identical in configuration because both limbs are more to the front of the heart than either is to the left or to the right of the heart.

For this reason, when a dog develops even massive left ventricular enlargement, QRS in all leads may appear normal. In man, when the left ventricle enlarges it causes the electrical axis to shift leftward and craniad; while, for the dog with left ventricular enlargement, the electrical axis remains oriented caudad. The voltages may be augmented in leads monitoring the cranio-caudal axis of the torso (II, III, and aV_F), but such wide variations in these voltages occur normally, one is hard pressed to differentiate normal from abnormal.

With use of either additional thoracic leads[27] or a corrected orthogonal lead system such as the McFee axial,[28] the electrocardiogram may provide more useful information about left ventricular enlargement than can be obtained currently.

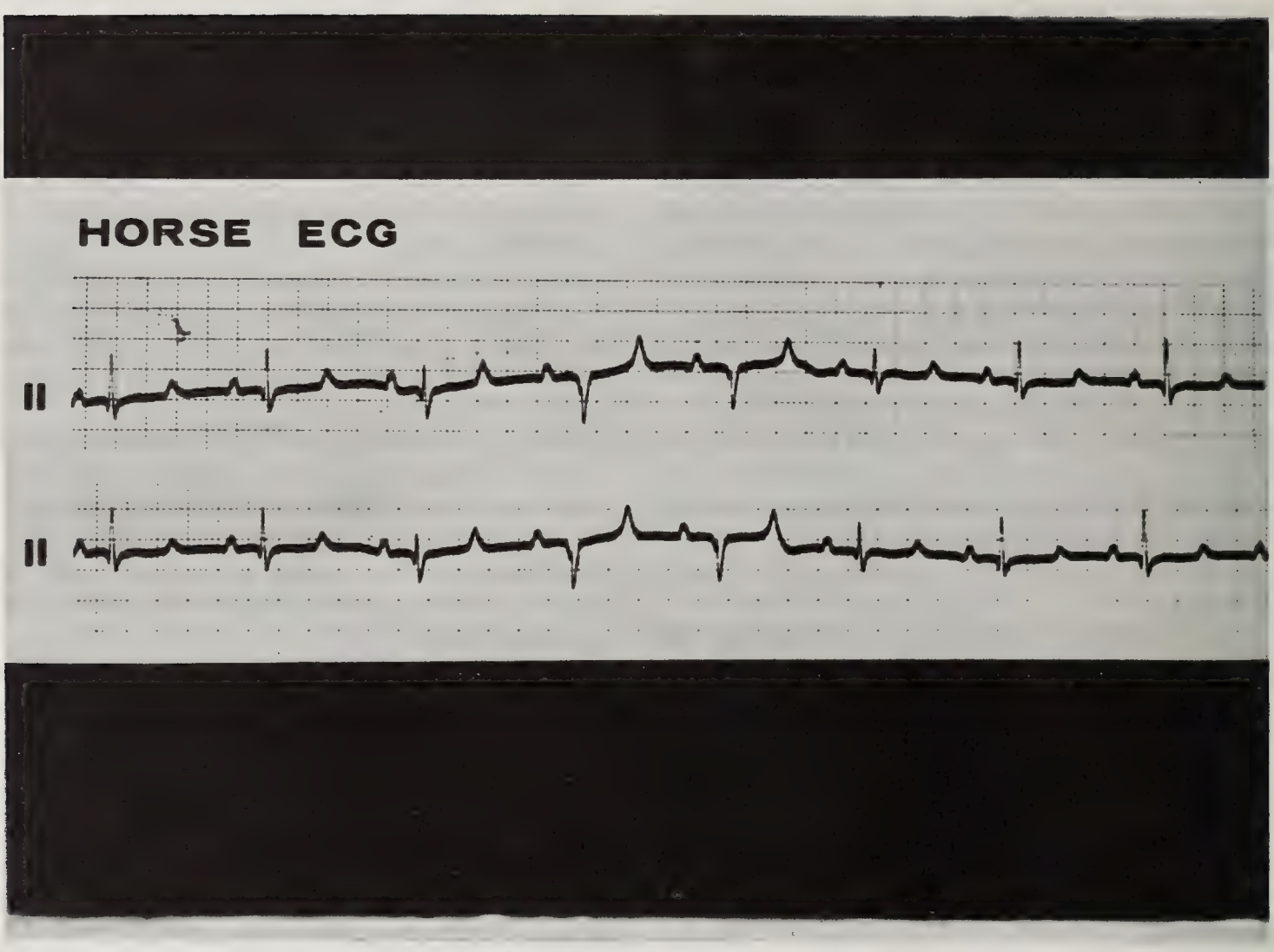


FIGURE 3.—Electrocardiogram lead II, monitoring the cranio-caudal axis, from a normal horse. Notice the changing configuration of the QRS complex in 3rd, 4th, and 5th complexes from the left. The R-wave becomes progressively lower in amplitude, changing from that observed in a dog to that observed in a goat.

Differences in forms of body surface potentials among the various species may be caused by differences in pathways of cardiac activation or differences in orientation of the heart within the torso. Another possible cause, might be alterations in the characteristics of the body as a volume conductor. It is interesting to speculate about the significance of voluminous air sacs in birds [29] and the pendulous rumen in goats and cattle and the cecum in horses. In any case, we feel that we can explain electrocardiographic differences on the basis of only the activation process and heart position.

SUMMARY

The electrocardiogram has serious limitations in studying abnormalities of the heart in certain species in which inordinate degrees of cancellation occur during ventricular depolarization.

If, in the pathogenesis of disease, alterations in ventricular activation are important, then animals with complete penetration of Purkinje fibers throughout both ventricular free-walls should not be used as models. No matter how severe the heart lesion, ventricular activation may be changed only minimally. However, these species may serve as useful models to study the effects of lesions independent from changes in ventricular activation.

Larger species are of interest because of their propensity for developing atrial arrhythmias.

Another interesting aspect of comparative electrocardiography lies with phenomena con-

sidered normal in certain mammals but considered to be abnormal in man.

Differences exist in postures within the thorax of hearts of quadrupeds compared with hearts of primates. Even though cardiac activation may be identical in certain quadrupeds and primates, body surface potentials of one may be grossly different from surface potentials of the other.

REFERENCES

1. Hamlin, R. L., J. Himes, H. Guttridge, and W. Kirkham. P wave in the electrocardiogram of the horse. Am. J. Vet. Res. 31:1027–1031, 1970.
2. Hamlin, R. L., D. L. Smetzer, T. Senta, and C. R. Smith. Atrial activation paths and P waves in horses. Am. J. Physiol. 219:306–313, 1970.
3. Lannek, N. A clinical and experimental study of the electrocardiogram in dogs. Thesis, Stockholm, 1949.
4. Alfredson, B. V. and J. F. Sykes. Studies on bovine electrocardiogram. II. Bundle branch block. Proc. Exptl. Biol. Med. 43:580–584, 1940.
5. Van Arsdale III, W. C., H. Krueger, and R. Bogart. Vector orientation of P, QRS, and T axis in beef cattle. Am. J. Vet. Res. 24:956, 1963.
6. Hamlin, R. L., S. Ridgway, and W. Gilmartin. Electrocardiogram of pinnipeds. Am. J. Vet. Res. (Accepted for publication in March, 1972).
7. Hamlin, R. L. and C. R. Smith. Categorization of common domestic mammals based upon their ventricular activation process. Ann. N. Y. Acad. Sci. 127:195–203, 1965.
8. Durrer, D. and L. H. Van der Tweel. Excitation of the left ventricular wall of the dog and goat. Ann. N. Y. Acad. Sci. 65:779–803, 1957.
9. Scher, A. M. Ventricular depolarization in the dog. Circulation Res. 4:461–479, 1956.
10. Scher, A. M., A. C. Young, and A. L. Malmgren. Multichannel recording apparatus for physiologic studies. Rev. Sci. Inst. 26:603–605, 1955.
11. Hamlin, R. L., D. L. Smetzer, and C. R. Smith. The electrocardiogram, phonocardiogram, and derived ventricular activation process of domestic cats. Am. J. Vet. Res. 24:728, 1963.
12. Robinson, F. R. and R. L. Hamlin. The electrocardiogram and vectorcardiogram of normal Macaca mulatta in dorsal-supine, right lateral, left lateral and sitting positions. Biomedical Laboratory Aerospace Medical Research Laboratories, ASD Technical Report 61–738, Dec. 1961.
13. Hamlin, R. L. and A. M. Scher. Ventricular activation process and genesis of QRS complex in the goat. Am. J. Physiol. 200:223, 1961.
14. Hamlin, R. L., C. R. Smith, and R. W. Redding. Time-order of ventricular activation for premature beats in sheep and dogs. Am. J. Physiol. 198:315, 1960.
15. Hamlin, R. L., F. S. Pipers, R. M. Kondrich, and C. R. Smith. QRS component of the orthogonal lead, spatial magnitude, and spatial velocity electrocardiograms, and vectorcardiograms of turkeys. J. Electrocardiology, 2(2):127–134, 1969.
16. Meyling, H. A. and H. Ter Borg. The conduction system of the heart in hoofed animals. Cornell Vet. 42:419–455, 1957.
17. Davies, F. The conducting system of the bird's heart. J. Anat. 64:9, 1930.
18. Hamlin, R. L., D. L. Smetzer, and C. R. Smith. Analysis of the QRS complex recorded from a semi-orthogonal lead system in the horse. Am. J. Physiol. 207:325–333, 1964.
19. Hamlin, R. L., R. Jackson, J. Himes, F. S. Pipers, and A. Townsend. Electrocardiogram of the bottle-nosed dolphin (Tursiops truncatus). Am. J. Vet. Res. 31:501–505, 1970.
20. Hamlin, R. L. The QRS electrocardiogram, epicardiogram, vectorcardiogram and ventricular excitation process of swine. Am. J. Physiol. 198: 537, 1960.
21. Redding, R. W. The spread of the excitation wave in the ovine left ventricle. Ph.D. Dissertation, Ohio State Univ., Columbus, Ohio, 1957.
22. Redding, R. W., C. R. Smith, and R. L. Hamlin. Spread of the excitation wave in the ovine left ventricle. Abst. 32nd Scientific Sessions, Am Heart Assoc., 1960.
23. Hoffman, B. F. and P. F. Cranefield. Electrophysiology of the Heart. New York, N. Y.: McGraw-Hill, 1960.
24. Hamlin, R. L. and C. R. Smith. A method for teaching genesis of the ECG and simulating effects of morphologic and conduction defects. Am. J. Vet. Res. 31:2289–2300, 1970.
25. Hamlin, R. L., F. S. Pipers, H. K. Hellerstein, and C. R. Smith. Alterations in QRS during ischemia of the left ventricular free wall in goats. J. Electrocardiology, 2(3):223–228, 1969.
26. Hill, J. D. The significance of foreleg position in the interpretation of electrocardiograms and vectorcardiograms from research animals. Am. Heart J. 75:518, 1968.
27. Hill, J. D. A correlative study of spontaneous right ventricular conduction disturbance in the dog. Thesis. University of Penn., 1967.
28. McFee, R. and A. Parungao. An orthogonal lead system for electrocardiography. Am. Heart J. 62: 93, 1961.
29. Douglas, S. D. Correlation between the surface electrocardiogram and air sac morphology in the white leghorn rooster. Am. J. Physiol. 199:355, 1960.

DISCUSSION

J. W. CLAYTON, University of Wisconsin, Madison: Today there is a great deal of concern about the use of aerosol products which in some cases are propelled by a class of materials known as the fluorocarbons. These are fluoroalkanes, and in some cases the dog is used to evaluate the effects of cardiac rhythm from inhalation of these materials. Would you comment on that please?

DR. HAMLIN: Yes, I think a dog is a particularly bad model to test the arrythmogenicity of any compound. Dr. Harris, why may be here in the audience, might care to comment about it. He has shown that if one stimulates the stellate ganglia of a dog that has been anesthetized with halothane, one will not produce an arrythmia. Whereas, if one stimulates that of a cat or, probably man, horrendous ventricular arhythmias may occur. So it appears that the dog, at least, might not be a very good model at all to study the sensitivity of a myocardium, particularly if that sensitivity is enhanced by autonomic efferent drive.

DR. CLAYTON: How about the monkey then?

DR. HAMLIN: I can't say about the monkey.

J. W. BUCHANAN, University of Pennsylvania, Philadelphia, Pa.: Dr. Hamlin, you might review for this audience some of the aspects of sinus arrythmia in the dog and how to account for it in studies.

DR. HAMLIN: It is very interesting when one analyzes the normal electrocardiogram of some of the species. One sees rather striking variations. And I think when one looks at what is considered normal for these animals, and might be considered abnormal for man, there probably is an interesting story. Sinus arrythmia is probably as pronounced in the dog as in any other species, possibly with the exception of some birds. The mechanism of it was of interest to us, as I'm certain it is to Dr. Levy, who is quite expert in the determination of sinus rate as a balance between autonomic nerves. We found that there are changes in RR intervals of the dog's electrocardiogram, varying between 300 and 1200 milliseconds. This waxing and waning of heart rate in the dog appears to be a function of vagal efferent activity. In the bird, on the other hand, it's been proposed that the fluctuations in heart rate are a sequel to fluctuations in sympathetic efferent activity, so when one studies the effects of blood pressure on the reduction or increase of heart rate, there might be some large species' differences.

DR. BUCHANAN: Another question. Would you comment on how to standardize limb positioning for dogs in repeat studies so that they don't encounter this variation in the electrocardiogram you mentioned with the limb position?

DR. HAMLIN: Surely. This is a very important problem and I think that there are two ways of getting electrocardiograms. One is where the trace jumps all over the place and the animal is unanesthetized and unrestrained and you try to look for gross changes. The other is where the animal is properly restrained—even if medically restrained. One can obtain certain standardizations if the limb leads and the neck are placed in the proper position. Dr. Hill at the University of Pennsylvania did an excellent study in which he showed that the thoracic limbs should come out at about right angles to the torso, that the position of the pelvic limbs was less important than that of the cranial limbs, and that the neck should be placed so that the Y-axis of the animal is fairly straight. That the limbs be placed in a consistent position is extremely important, as Dr. Buchanan suggests, in interpreting the electrocardiogram of any quadruped.

W. C. VAN ARSDEL, Food & Drug Administration, Washington, D.C.: The electrical axes of the QRS&T in some species are a little different. Have you gone into that?

DR. HAMLIN: The next parameter we were going to add to our characterization of these animals is the T-wave. Unfortunately, the T-wave, as Dr. Lepeschkin knows, is like a chameleon in many of these animals. It's very labile and in some of the species, not only is it labile, but it's multiphasic. For example, the horse has a polyphasic T-wave; and because of its liability, it's pretty hard to characterize it accurately. Although one can get a consensus, I really wouldn't care to talk about it now because it's something on which we have so little information.

K. T. LEE, Albany College of Union University, Albany, N.Y.: What are the differences in electrocardiograms of human and swine? We've been doing some swine electrocardiograms and I think there are some differences. Do you have some experience?

DR. HAMLIN: Yes, we find that the pig lies somewhere between the ruminant and the carnivore. And we place it approximately in the IIB category, along with the horse and the dolphin. People have made the analogy that the dolphin is akin to a swimming pig. If one looks at the axis, (the anterior posterior or dorsao ventral axis), one finds that in pigs the mean and major forces are directly dorsal, (that is to the back); as opposed to man, whose forces might be 50% anterior and 50% posterior. So I think there are rather striking differences in swine as compared with man, but swine fit very closely with horses and dolphins. The pig may be an unfortunate animal in some cases because he has this nearly completely penetrating Purkinje system. If the experiment you're conducting depends upon studying the hemodynamic consequences of this conduction system, say you're forcing the heart with some pathological means, that's one thing. But, if it depends upon conduction disturbance, then it's quite another, because the pig is much less likely to get significant conduction disturbance because of this anastomosing Purkinje system.

E. F. JANKUS, University of Minnesota, St. Paul: It's a tremendous job to try and classify these various conduction routes on a species' basis. I wonder if you would comment on Davies' report in the 1930's about the Purkinje distribution via the coronary arteries.

DR. HAMLIN: Davies did propose that the Purkinje system of birds penetrates from epicardium to endocardium. I'm certain that it does. Whether this produces any significant difference between that of the bird and the cow is questionable, because in any case, it seems to penetrate the entire transmural distance, and one can account for the activation process similarly as one does with the cow. That is, it doesn't matter (if the ventricular free walls are excited with a burst of activity) where that activity begins, whether the Purkinje fiber penetrates from endocardium to epicardium or epicardium to endocardium. This is something that we're currently investigating. Dr. Moore has looked at it in birds and could reflect better on the subject.

DR. BUCHANAN: I was puzzled by your diagrams. Could you clear up the nomenclature of anterior-posterior as opposed to dorso-ventral?

DR. HAMLIN: The Z axis is the anterior posterior or dorsal ventral and the Y axis is the cranial caudal or superior inferior.

DR. BUCHANAN: Considering the axes on the quadruped animals, they're like the bovine, it didn't look like you had much QRS potential. Now, what were the spike potentials there?

DR. HAMLIN: There is some activity. Since most of the activity ascends the septum in these animals, one is most likely to get greatest voltages in the anterior posterior axis and very small voltages in the cranial caudal axis.

DR. BUCHANAN: I was just wondering which way because you didn't seem to have enough potential.

DR. HAMLIN: There are very small voltages in the cranial caudal axis of these animals.

W. B. WEAD, University of Louisville, Louisville, Kentucky: I was wondering if you have a VCG analysis, comparative analysis, and if you have, does it hold up under this type of division? What is the best lead system that you've come up with?

DR. HAMLIN: We have done vector cardiographic analyses, and we use the McFee axial system. We have tried the Frank system as well, and there's apparently very few differences between the McFee axial and the Frank. The voltages are a little higher in the McFee than in the Frank, and they correlate very closely with the unipolar thoracic leads. There are some local effects, some proximity effects, that one sees with a thoracic lead.

DR. WEAD: Does the position of the McFee leads still matter as much in these animals as it does in man as opposed to the Frank lead system with which you have little bit more leeway.

DR. HAMLIN: I can't say much about the comparison between man and the quadrupeds. It appears to be rather insensitive to position in the quadrupeds.

JACK HAN, Albany Medical College, N.Y.: I'm under the impression that the refractory period of a bundle branch system in swine is much longer than that of the A–V node. We have done some His bundle electrograms, and when we stress, say, the A–V conduction system in swine, we have found that the level of block is below rather than above the His. I am wondering if this may explain the unusually long acute interval in swine which we have also observed?

We also find that the fibrillation threshold is very much lower in swine than in the dog. This may have some relationship to a long acute interval in swine, too.

DR. HAMLIN: Those are interesting comments. It is my impression, however, that in all species the duration of the longest action potential is below the A–V junction in the bundle of His, or approximately the left or right bundles. So I think the pig would be consistent with that.

PHYSIOLOGIC AND PHARMACOLOGIC RESPONSE OF CHRONICALLY DENERVATED FELINE HEARTS

Kenneth M. Kent, Peter J. Dempsey,
Zena T. McCallum and Theodore Cooper*

The terminal sympathetic nervous supply to the heart can be abolished by a technique of mediastinal neural ablation. As degeneration of the nerves occurs, the catecholamine stores of the heart are depleted. Fifty-four cats were subjected to extrinsic cardiac denervation through a right lateral thoracotomy under halothane anesthesia. Three to four weeks following the operation the hearts were removed and the isolated cardiac tissue was studied. The function of the whole heart was assessed on a Langendorff apparatus, and right ventricular papillary muscles were studied in a myograph. At the time of study the hearts contained less than 0.01 μgm of norepinephrine per gram of heart. The transmembrane action potential was recorded in some experiments by the use of KC1 filled microelectrodes which had a tip diameter of approximately 2 microns. The isolated cardiac tissue following denervation was supersensitive to norepinephrine, and this augmentation was felt to be a result of decreased reuptake of the humoral agent by the sympathetic nerve terminals. The response of the catecholamine depleted cardiac muscle to hypoxia was different from control cardiac tissue. When normal papillary muscles were subjected to hypoxia, tension and rate of tension development decreased. When catecholamine depleted muscles were subjected to the same degree of hypoxia, the decrease in contractility was less than that of the control muscles. Also, hypoxia changed the transmembrane action potential so that the plateau (Phase 2) was shortened. This occurred to the same degree in control and catecholamine depleted muscles.

INTRODUCTION

The technique of extrinsic cardiac neural ablation made possible the study of the physiological adaptation of chronically denervated hearts,[1] the pharmacological interactions of the neural mediators,[2,3] the trophic influences of the cardiac nerves[4] and the direct myocardial action of pharmacological agents which also release myocardial norepinephrine.[5-7]

The sympathetic nerve fibers enter the heart around the great vessels as well as with the vagal cardiac nerves.[8] The destruction of the sympathetic postganglionic nerve fibers as they enter the heart causes degeneration of the myocardial nerve fibers and loss of the adrenergic nerve terminals and the adrenergic neural mediator—norepinephrine. The ganglia of the cholinergic system, however, lie within the heart, and severing the vagal cardiac fibers removes the preganglionic fibers only. The cholinergic ganglia and the postganglionic fibers within the heart itself remain intact after extrinsic cardiac denervation.

METHODS

Extrinsic cardiac denervation was performed in the following manner. Male cats, 2.5 to 3.5 kg, were given 0.4 mg atropine sulfate i.m., placed in a restraining bag, and administered fluorothane 3% (Fluotech 3) in oxygen by mask. When surgical anesthesia was reached, the animals were given succinylcholine 4 mg i.v. and the trachea was intubated. With the animals on positive pressure respiration (Harvard Instruments) at a tidal volume of 75 ml, anesthesia was maintained with ½ to 1% fluothane in a closed system. A right thoracotomy in the fifth intercostal space was performed under sterile conditions. The azygos vein was cauterized and cut. The right vagus and phrenic nerves were dissected and cleared from adjacent tissue from the thoracic inlet to the diaphragm for the phrenic nerve and to the azygos vein for the vagus nerve. The cardiac branches of the right vagus nerve which usually came off of the main trunk dorsal to the azygos vein were identified and cut. The thymus gland was removed in its entirety by dissection and cauterization. The pericardium was opened. The aortic adventitia was stripped circumferentially at the root of the aorta. The aorta and aortic arch vessels were then stripped of their adven-

* National Heart and Lung Institute Bethesda, Maryland 20014.

titia and separated from the main pulmonary artery and its branches. Electrocautery was used for most of this dissection to further aid in the destruction of nerve fibers.

The left phrenic and vagus nerves were identified across the mediastinum and dissected in the same manner as on the right side. The left vagal cardiac branches were identified and cut. The superior vena cava was isolated. At this point all of the structures which had previously been identified—both vagus and phrenic nerves, aortic arch and its branches, pulmonary artery and its branches, and superior vena cava, esophagus and trachea—were retained, and the remaining structures in the superior mediastinum were ablated with electrocautery. The pericardium was cut dorsally on the left side down to the left atrium. Careful hyperinflation of the lungs was performed, and the chest was closed. After closure of the chest 10 to 30 ml of air were evacuated with a blunt 16 gauge needle carefully inserted in the sixth to eighth intercostal space. The animal was given 50 ml of balanced salt solution subcutaneously and allowed to recover from general anesthesia. Benzathine penicillin 300,000 units and procaine penicillin 300,000 units were given intramuscularly on the operative day, and 5 days later 300,000 units of benzathine penicillin was given. Nitrofuazone powder and an aerosal spray bandage was applied postoperatively. Occasionally gastrointestinal complications were evident in the postoperative period which probably were a result of vagal nerve manipulation. Therefore no attempt was made to feed the animals until 36 to 48 hours postoperatively. Rarely, feeding had to be started with a gastric tube on the fourth postoperative day.

Catecholamine stores of the heart were determined by the trihydroxy indole method.[9]

Isolated Whole Heart

Adult male cats, 2 to 3.5 kg, were anesthetized with sodium thiamylal (Surital) 30 mg/kg intrapleurally and their hearts were removed quickly and mounted on a cannula. Retrograde perfusion of the coronary arteries was carried out at a constant flow rate (20 cc/min) with a modified Krebs-Ringer solution of the following composition per item: Na^+, 146 mM; K^+, 3.6 mM; $H_2PO_4^-$, 1.2 mM; Ca^{++}, 2.5 mM; Mg^{++}, 1.2 mM; Cl^-, 128 mM; $SO_4^=$, 1.2 mM; HCO_3^-, 25 mM; and glucose, 5.6 mM. The pH was 7.4, and the temperature was 34 ± 0.5° C; the solution was oxygenated with 95% O_2 − 5% CO_2. Fluid was not recirculated.

Mean perfusion pressure was monitored with a Statham P23 Db strain gauge through a side arm on the perfusion cannula and varied from 25 to 32 mm Hg. Peak pressure developed by the isovolumically beating left ventricle was used as an index of contractility. For this purpose a small latex rubber balloon mounted on an 18-gauge cannula was introduced through a purse-string suture into the left ventricle and filled with a small amount of saline. The pressure developed in the balloon was measured by means of a Statham P23Db strain gauge. A previous study demonstrated that within a wide range, variation in balloon pressure was not an important determinant of the percent change of the observed responses.[2] The first derivative of the pressure tracing (dp/dt) was monitored on-line by means of an active R-C circuit differentiator with a time constant of 1 msec. The perfusion pressure, balloon pressure, and dp/dt were recorded simultaneously on a direct writing oscillograph. Heart rate was maintained constant by right ventricular pacing.

With the perfusion pressure monitored closely, these preparations were stable for up to three hours, although the experiments reported seldom extended over two hours. The reactivity of the Langendorff preparation was verified by reproducibility of dose-response curves to standard inotropic agents, such as calcium after varying lengths of time.

Isolated Papillary Muscle

The cats were anesthetized in the same manner as above, the hearts were quickly removed, and right ventricular papillary muscles (0.9 to 2.2 mm in diameter) were removed after the tendinous end was tied with 5–0 Mersiliene suture. The muscles were then mounted in a 25 ml flow-through Lucite bath. The cut mural end was inserted in a spring loaded clip which was attached to a Minneapolis Honeywell #762025 isometric force-displacement transducer. The tendinous end was tied to a fixed post in the

bath. Modified Krebs-Ringer solution of the same composition as cited above was equilibrated at 25°C with a 95% O_2 and 5% CO_2 gas mixture in a remote column. The pH of the solution was 7.4. The Krebs was circulated to the muscle bath from the column by gravity and a return pump. The muscles were stimulated by two fine platinum electrodes near the base of each muscle at 12/min and approximately 5% above threshold voltage. The resting tension of the muscle was the tension at the peak of the length-tension curve. The first derivative of the tension (dT/dt) was obtained on-line.

For the hypoxia studies, after control tension measurements were made, the equilibrating gas mixture was changed to 5% O_2, 5% CO_2, and 90% N_2. The pO_2 of the Krebs solution was recorded in the following manner: 0.3 ml/min of the bath solution was suctioned from a port in the bath near the muscle and directed to a Lucite cell which held a Clark oxygen electrode. The electrode was calibrated before and after each experiment with known gas mixtures. The pO_2 was continuously recorded on an oscillograph.

Two muscle preparations were studied simultaneously in identical muscle baths. The protocol of the experiment was as follows: The muscle was first bathed in Krebs solution under high oxygen tension. Developed tension and dT/dt at L_{max} were determined after one hour of equilibration. The equilibrating gas mixture was then changed to the low oxygen one and developed tension and dT/dt were measured after one hour in hypoxia.

Tension, dT/dt, and pO_2 were continuously recorded on a Sanborn 350 direct writing oscillograph. Tension development and dT/dt were expressed as per cent change from baseline.

Transmembrane Action Potentials

Action potentials were recorded by means of 3M KCl-filled glass microelectrodes with a tip resistance of 5 to 20 megohms. The recording system consisted of a Bak standard wideband electrometer (Electronics for Life Sciences) and a Tektronix model 502 dual beam oscilloscope. All data during an experimental run were recorded on a multichannel Hewlett-Packard 3955 tape system.

RESULTS

Twenty-one days following the operative procedure, the tissue catecholamine content was at its minimum < 0.01 μgm/g tissue. At that time there was no response to tyramine.

Interactions of Neural Transmitters

The suprasensitivity of chronically denervated feline hearts was previously described in our laboratory.[3] When norepinephrine (NE) was administered to isolated whole heart preparations, the responses to a given dose of NE were greater in the chronically denervated hearts than in the hearts from control animals (Figure 1).

Responses to Hypoxia

Right ventricular papillary muscles when subjected to hypoxic perfusing solutions demonstrated decreased contractility. As the pO_2 of the Krebs solution was decreased to 120 mm Hg (5% O_2) the developed isometric tension decreased 32 ± 3% (average ± SEM) in control myocardium. However, the developed tension of

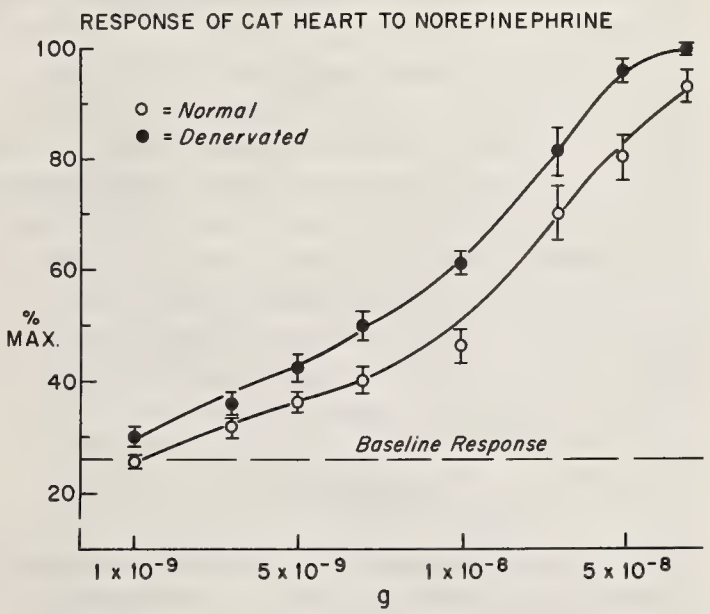


FIGURE 1.—Dose-response curves for norepinephrine in the normal versus denervated feline heart. The percent of maximum response for the isolated whole heart is shown as a function of the norepinephrine dose in grams. The mean responses and ± SEM are shown for normal (●) and chronically denervated (o) hearts. This figure is reproduced with the permission of American Physiology Society.

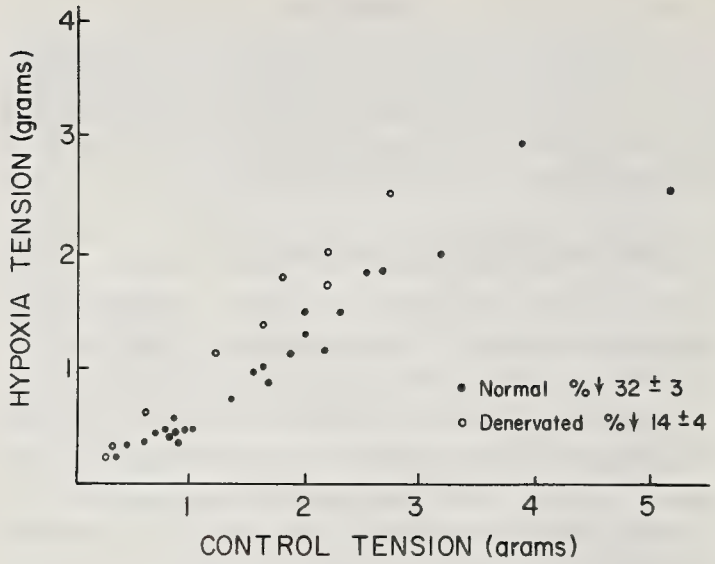


FIGURE 2.—Developed isometric tension in hypoxia as a function of tension under control (oxygenated) conditions.

chronically denervated papillary muscles fell only 16 ± 4%. Figure 2 illustrates the developed tension in hypoxia as a function of the control tension. The responses of papillary muscles from chronically denervated cats are shown. The time to peak tension (TTP) decreased in the control muscles as hypoxia was induced 0.48 ± 0.03 msec to 0.39 ± 0.02 msec ($p < 0.05$). However, there were no changes in TTP in the muscles from chronically denervated cat hearts 0.43 ± 0.02 to 0.42 ± 0.03 msec ($p < 0.1$). The effects on the shape of the transmembrane action potential (TAP), however, were similar for the control and the chronically denervated papillary muscles (Figure 3). Although there was a slight decrease in the resting potential of this example, this decrease was not a constant finding.

DISCUSSION

Myocardial catecholamine depletion by extrinsic cardiac denervation has elucidated the complex interaction of the neural mediators acetylcholine and norepinephrine.[2-4] Catecholamine depletion has also made it possible to separate the direct myocardial actions of bretylium,[5] angiotensin II,[6] analogues of angiotensin II (10), and ouabain[7] from possible indirect-NE releasing properties.

As the myocardial adrenergic terminals degenerate, the properties of binding, reuptake and degradation of norepinephrine are lost. Therefore, in denervated hearts exogenously administered NE is inactivated more slowly, a change that causes larger responses to the norepinephrine.

Responses to hypoxia of chronically denervated hearts are significantly different from control animals. The decrease in tension caused by hypoxia is less in the chronically denervated-catecholamine depleted heart muscle. The mechanisms responsible for this difference are unclear. Scheur and Stezoski[11] have demonstrated that short-term reserpine pretreatment has a protective effect in rat hearts subjected to hypoxia. They hypothesized that this effect of reserpine was related to the increased glycogen content of the reserpinized myocardium (the dose schedule of reserpine used produced only small changes in norepinephrine content). Similarly, there is an increased glycogen content of denervated canine hearts.[12]

Trautwein et al.[13] first demonstrated the effects of hypoxia on the transmembrane action potential—a decrease in the plateau phase

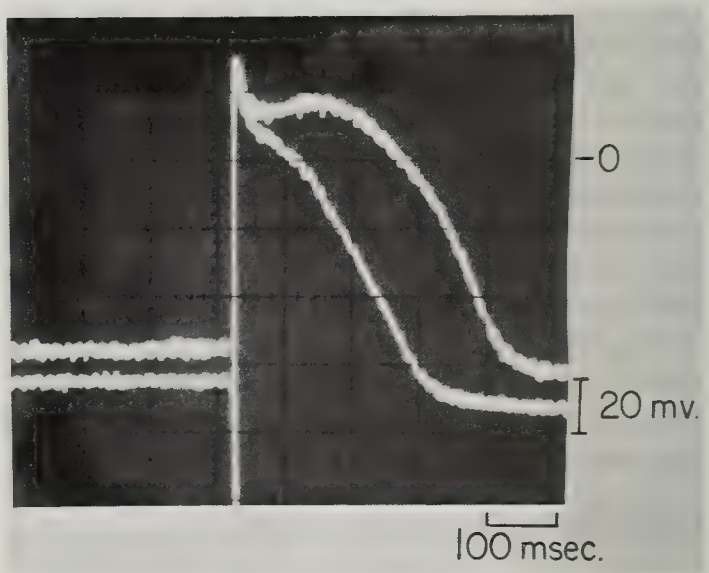


FIGURE 3.—Transmembrane action potential (TAP) of an isolated papillary muscle from chronically denervated cat heart. The two curves are superimposed tracings of the TAP in control conditions—curve to the right—and the shortening of the plateau and decreased slope of repolarization caused by hypoxia. The resting potential decreased slightly in hypoxia in this example, but the resting potential was usually not affected by this degree of hypoxia.

(phase 2). Also Bassett et al.[14] have demonstrated a diminution of the rate of rise of phase 0 as canine Purkinje fibers were made hypoxic. Although the contractile responses of the catecholamine-depleted hearts are less impaired by hypoxia than the control muscles, the same degree of shortening of phase 2 of the TAP occurred in all muscles studied, control and denervated. Thus, there appears to be a dissociation of the membrane effects of hypoxia and the effects on contractility in catecholamine-depleted myocardium.

SUMMARY

The technique of extrinsic cardiac denervation in cats has made it possible to study catecholamine-depleted isolated whole hearts by using a Langendorff apparatus as well as isolated right ventricular papillary muscles. In addition to contractility studies, it is possible to assess the transmembrane action potential of the chronically-denervated myocardium.

REFERENCES

1. Donald, D. E., and Shepard, J. T. Response to exercise with cardiac denervation. Am. J. Physiol. 205:393–400, 1963.
2. Dempsey, P. J., and Cooper, T. Supersensitivity of the chronically denervated feline heart. Am. J. Physiol. 215:1245–1249, 1968.
3. Dempsey, P. J., and Cooper, T. Ventricular cholinergic receptor systems. J. Pharmacol. Exp. Ther. 167:282–290, 1969.
4. Guth, L., Dempsey, P. J., and Cooper, T. Maintenance of neurotrophically regulated protein in denervated skeletal and cardiac muscle. Exp. Neurology 32:478–488, 1971.
5. Gaffney, T. E., Braunwald, E., and Cooper, T. Analysis of the acute circulatory effects of guanethidine and bretylium. Circulation Res. 10:83–88, 1962.
6. Dempsey, P. J., McCallum, Z. T., Kent, K. M., and Cooper, T. Direct myocardial effects of angiotensin II. Am. J. Physiol. 220:477–481, 1971.
7. Dempsey, P. J., McCallum, Z. T., Kent, K. M., and Cooper, T. Dissociation of cardiac inotropic and transmembrane action potential effects of ouabain. J. Pharmacol. Exp. Ther. 177:78–84, 1971.
8. Hirsch, E. F., Kaiser, L. C., Barnes, H. C., and Cooper, T. The innervation of the feline heart. In *The Innervation of the Vertebrate Heart.* Ed. E. F. Hirsch. Charles C. Thomas, Springfield, Ill. 1970, pp 64–80.
9. Crout, T. R. *Standard Methods in Clinical Chemistry.* Ed. D. Seligson. New York Academic Press. 1960, vol. 3 p 62.
10. Kent, K. M., Goodfriend, T., McCallum, Z. T., Dempsey, P. J. and Cooper, T. Inotropic agents in hypoxic cat myocardium: Depression and potentiation. Circulation Res.
11. Scheur, J., and Stezoski, S. W. Protective role of increased myocardial glycogen stores in cardiac anoxia in the rat. Circulation Res. 27:835–849, 1970.
12. Jellinek, M. P., Kaye, C., Nigh, A., and Cooper, T. Alterations in chemical composition of canine heart after sympathetic denervation. Am. J. Physiol. 206:971–974, 1964.
13. Trautwein, W., Gottstein, U., and Dudel, J. Der Aktionsstrom due myokard faser im Sauerstoffmangel. Arch ges Physiol. 260:40, 1954.
14. Bassett, A. L., Bigger, J., Jr., and Hoffman, B. F. "Protective" action of diphenylhydantoin on canine purkinje fibers during hypoxia. J. Pharmacol. Exp. Ther. 173:336–343, 1970.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. Joseph Pierce for his assistance in the development of the operative procedure in cats. We also appreciate the technical assistance of Mr. James Hopkins and Mr. Everett Hodge during the operative procedure.

DISCUSSION

J. B. Senturia, Cleveland State University, Ohio: There exists an animal model for this system that you're talking about in nature: the hibernator, which would stand hypoxia. Animals like the European hedge hog, ground squirrel and so forth seem to lack adrenergic innervation to the myocardium. There's been some dispute about whether the data is completely correct or not, but experiments by Ouveman and Nielson in Sweden seem to indicate that this is exactly the case. They do lack adrenergic innervation normally, and, of course, they withstand hypoxia considerably. All of the hibernators seem to be able to withstand hypoxia; so there is a natural animal model for this system that does exist.

Dr. Kent: That's very interesting. The hibernator, of course, physiologically during hibernation is probably not hypoxic, but certainly they do stand hypothermia, hypoxia, and a lot

of stresses much better. It's interesting. I didn't know about the sympathetic innervation.

E. LEPESCHKIN, University of Vermont, Burlington, Vermont: How was hypoxia produced? Was it in a nitrogen saturated atmosphere or was the profusion discontinued?

DR. KENT: The gas for the control studies was 95% oxygen and 5% CO_2. We maintained the hypoxia studies at 5% oxygen, 5% CO_2, and the balance was nitrogen. There was only lowered oxygen tension.

M. N. LEVY, Mt. Sinai Hospital, Cleveland, Ohio: Carrier, Barger, et al, and Buccino and Friedman have described a very pronounced positive inotropic effect on the ventricular myocardium with acetylcholine in animals in which the myocardial catecholamines have been depleted with reserpine, or have been treated with beta blocking agents. Have you seen anything of that sort in your preparations, either in the normal or in the denervated preparation? Do you think that might represent a response that's at a completely different dose level?

DR. KENT: The work of Friedman that you quoted was in the papillary muscle. We have not actually carried out those experiments in the denervated papillary muscles. They were working at room temperature with slowly beating papillary muscles, and it could be just a difference in temperature and a difference in rates. In one of the earlier slides, we saw a small positive response to acetylcholine. I'm not sure why the difference exists, except that the experimental conditions were quite different. We've also seen a late positive inotropic response when we blocked the ability of the heart to release norepinephrine with cocaine, for example. Cocaine treatment of the normal heart will block its release of norepinephrine. So, with high doses of acetylcholine in a cocainized preparation, we get no early response, but then we get a response several minutes later. I am not sure of the exact mechanism of that. So, I don't think it's necessarily as simple as we've described here.

J. V. LEVY, Presbyterian Hospital, San Francisco, California: With regard to rate dependency, in isolated preparations, is there any difference in the force frequency relationship between the normal and the denervated preparation?

DR. KENT: No. That's been carefully studied now, and there is no difference. The only requirement is that the force frequency relationship be carried out with a threshold voltage of stimulation because a higher voltage causes norepinephrine release from the normal muscles. There will then be a difference then because the chronically denervated preparation won't perform as well as the normal.

J. P. ELLISON, Tulane University, New Orleans, Louisiana: Firstly, since you use eventually an isolated preparation, I wouldn't see any profound objection to attempting the sympathetic denervation with 6-hydroxydopamine rather than direct neuralization. Have you compared this?

DR. KENT: We have not. That's certainly an interesting tool that deserves much more study.

DR. ELLISON: The second point is that this residual population of postganglionic parasympathetic ganglion cells that you have, is, I believe, initially responsive both to cholinergic and adrenergic preterminals, and I wonder if this population is viable? I suppose it is, and I wonder how its responses might influence your results?

DR. KENT: The postganglionic cholinergic fibers can be stimulated. In the interactions we've thus far looked at, I can't see any possibility of their influencing our results.

"PACING" LEFT VENTRICULAR FUNCTION CURVES IN CONSCIOUS DOGS

F. Kraft-Hunter and E. W. Hawthorne*

In seeking to determine the efficacy of using "pacing" ventricular function curves, obtained from conscious instrumented animals, as one means of evaluating ventricular performance changes that might occur in them as a result of some experimental intervention, observations have been made of the effects of atrial, ventricular, and sequential pacing in dogs. The left atrium and ventricle were paced for short periods at 2, 2.5, 3, and 3.5 Hz using bipolar electrodes. Then sequential pacing using an atrial systole-ventricular systole of 95 milliseconds was used. The animals were instrumented so as to permit simultaneous recordings of d lnP/dt, dP/dt, aortic, left ventricular and atrial pressures, left ventricular internal diameter, and the left ventricular electrocardiogram. The recording of these primary variables permitted the calculation for each of a series of cardiac cycles, derived variables such as left ventricular volumes, stroke work, total peripheral resistance, stroke power, cycle lengths and a variety of other variables. A small digital computer and A to D converter was used for the on-line data acquisition and reduction. End-diastolic volume stroke work function curves were constructed using the data derived from the stepwise atrial pacing. It was observed that myocardial contractility, as evaluated by changes in peak dP/dt, developed left ventricular pressure (40 mm Hg), mean V_{CF} at the endocardial surface of the left ventricle, peak d lnP/dt and peak dP/dt, increased systematically with each increase in heart rate during atrial and sequential pacing. This was not seen with ventricular pacing. These "pacing" ventricular function curves were shifted to the left during periods of norepinephrine infusion and to the right after beta blockade using propranolol. Left ventricular pacing appears to provide usable left ventricular function curves in conscious instrumented dogs. It is suggested that this type of "pacing ventricular function curve" is one way to evaluate ventricular performance in man.

INTRODUCTION**

Until recently the Frank-Starling ventricular function curve[1,2] as clarified and popularized by the studies of Sarnoff and his associates[3-5] was viewed as the ultimate tool for evaluating ventricular performance. The difficulties with applying this approach to man are numerous. First there is the problem of the sensitivity of shift of these curves in demonstrating small changes in myocardial contractility. There is the difficulty of holding constant heart rate, aortic pressure, and myocardial catecholamine concentration in conscious man and animals so as to study the heterometric autoregulatory phenomenon through observing the effects of increasing venous return on the end-diastolic volume/stroke work relationship. Finally there is the matter of a reasonable, feasible and reproducible means of changing end-diastolic volume, and measuring the latter simultaneously with stroke volume and pressures in conscious man. During recent times the popularity for using the "force-velocity-volume relation"[6-9] in characterizing the heart as a muscle, and in terms of basic mechanics, has done much to deemphasize the use and the development of new and provacative approaches to the application of ventricular function curves in the routine analysis of cardiac performance in patients. Interestingly all during this period of renaissance atrial pacing has become commonplace for the cardiac patient. It is used for the treatment of selected atrial and ventricular arrhythmias. Atrial pacing has become a valuable tool for clinical and experimental investigation of electrocardiographic, metabolic and hemodynamic phenomena as they affect ventricular function. Recently, Desanctis[10] has discussed the diagnostic and therapeutic uses of atrial pacing as well as its safety. The concept of using "pacing" left ventricular function curves has thus emerged and served to revitalize, at least for a time, more intensive use of the ventricular function curve, and the concepts which must surround

* Department of Physiology & Biophysics, College of Medicine Howard University, Washington, D.C.

** This research was supported by Grant HE-01015-20.

its use, as a means of following and early detection of myocardial dysfunction.

Linhart[11] has put in one short paragraph the essence of what ventricular function curves are all about, and therefore his statement is quoted: "The ventricular function curve relating end-diastolic volume or filling pressure (preload) to the external work performed by the heart (stroke work or volume) has generally been used when characterizing the pumping action of the heart.[3-5] As end-diastolic volume and, therefore, end-diastolic fiber length increase up to a certain limit, the normal heart responds by a significant enhancement in its force of contraction (Frank-Starling mechanism)[1,2]. This is represented on the ventricular function curve by a large increase in stroke volume or work relative to small changes in end-diastolic volume or pressure. A change in initial fiber length, such as is caused by changes in heart rate (pacing) or afterload (angiotensin or methoxamine infusion,[12] will shift the ventricular response to various points on the same curve, indicating that intrinsic contractility has remained constant. Sarnoff has shown, however, that the ventricle has a family of ventricular function curves (VFC), and that the response may be shifted from one curve to another by enhancement or depression of myocardial contractility.[4] An increase in contractility is indicated by a shift of the VFC upward and to the left of the control state; a depression of function is indicated by a shift downward and to the right."

There have been many reports[13-35] describing the hemodynamic consequences of changing the rate of ventricular contraction. Ventricular rate has been changed by electrically pacing either the right atrium, left atrium, right ventricle or the left ventricle. Sequential pacing has also been used. Interestingly, most of these studies have been conducted in patients and normal human subjects. Some experimental studies have been made in anesthetized dogs and only a few have been made in conscious experimental animals.

Electrodes for pacing have been placed at numerous sites on both the ventricles and the atria in order to study the effect of pacemaking site on cardiac output. Finney[13] showed that ventricular function was significantly diminished with ventricular pacing as compared to atrial pacing. Changes in left ventricular peak dP/dt, peak left ventricular pressure, cardiac output, stroke work and stroke power for any given pacing rate were all found to be significantly greater when left ventricular pacing was used instead of right ventricular pacing. The reduced left ventricular function with right ventricular pacing apparently was not due to change in myocardial contractility, heart rate, end-diastolic fiber length or changes in compliance. The author suggested that dyssynergy of ventricular contraction had played an important role in this phenomenon.

Klotz, Lister, Jomain, Hoffman and Stuckey[14] also demonstrated in their studies that cardiac output was greater with pacing from the left ventricle than from the right. On the other hand Starzl, Gaertner and Webb,[15] William-Olsson and Anderson,[16] Benchimol and Liggett,[17] and Fletcher, Theilen, Lawrence and Evans[18] have found that at a given heart rate the site of ventricular stimulation did not influence either cardiac output or arterial pressure. It now seems clear that, regardless of pacemaking site, cardiac output is not significantly affected by changing heart rate, especially 80 to 180 beats per minute.[17] The studies of Stein, Damato, Kosowsky, Lau, and Lister[19] are illustrative of this point. They showed in 1966 that there was no significant change in the cardiac index when the heart rate was increased by atrial pacing in ten normal male volunteers ranging from 25 to 45 years of age, either at rest or during exercise.

All studies agree that there is an almost linear decrease in stroke volume with increases in heart rate. There is also general agreement that left ventricular stroke work decreases with increasing heart rate.

Gilmore, Sarnoff, Mitchell and Linden[20] showed, using anesthetized dogs, that when the ventricle is caused to contract as a result of direct electrical stimulation, it produces less stroke work from any given left ventricular end-diastolic pressure than when atrial stimulation is used at that same frequency. They observed that at any given left ventricular pacing rate, left ventricular end-diastolic pressure was lower and the left ventricle produced less exter-

nal stroke work than when the atrium was stimulated at the same frequency. This was believed to be largely because the ventricle was deprived of the atrial contribution to ventricular filling during ventricular pacing. These investigators also suggested that electrical stimulation of the left ventricle, in contrast to stimulation by a normally propogated impulse, resulted in the ventricle producing less stroke work from any given end-diastolic pressure, largely because of a less synchronous ventricular contraction. On the contrary, Benchimol and Liggett[17] found no significant differences between artificial stimulation of the right atrium and right ventricle at rates between 80 and 180 beats per minute. They observed no significant differences for cardiac index, stroke volume, mean arterial pressure, ventricular power and ejection time when the effect of atrial pacing was compared to ventricular pacing at any given rate between 80 and 180 beats per minute in normal subjects and in patients with either compensated heart disease or heart failure. They concluded that the contribution of atrial systole to cardiac function was minimal in normal subjects.

All studies show that end-diastolic volume decreases with increase in pacing rate. In 1966 Glick, Williams, Harrison, Morrow and Braunwald[21] sutured Roentgen opaque markers to the surfaces of one or both ventricles in nine patients at the time of corrective cardiac surgery. Post operative cineradiograms were obtained and the changes in the distances between the markers were measured at regular periods throughout several heart cycles. When heart rate was increased by atrial pacing (by an average of 56 beats/min), they noted that right ventricular end-diastolic and end-systolic dimensions decreased by averages of 7.2 and 4.9%, respectively. Left ventricular end-diastolic length decreased an average of 6.0%.

These observed effects of heart rate on ventricular size are of course not new revelations. Since the beginning of this century it has been appreciated that heart rate may modify the size of the ventricles. Yandell Henderson in 1906[22], using a cardiometer placed about an open chest dog's heart observed that both the end-diastolic and end-systolic size of the whole heart decreased as heart rate was increased. Furthermore, he found that end-diastolic size decreased more than did end-systolic size for any given heart rate increase. Patterson, Piper and Starling[23] using their now famous heart-lung preparation and Wiggers and Katz[24] observed that slowing the heart rate by vagal stimulation increased both end-diastolic size and stroke volume. This greater fall in end-diastolic size than end-systolic (and therefore decrease in stroke volume) was also observed in 1960 by Chapman[25] and by Miller, Gleason, Whalen, Morris and McIntosh[26] in 1962 in studies involving the use of cineangiographic techniques. Rushmer[27] noted that the left ventricular external diameter of conscious dogs decreased with each increase in rate of right atrial stimulation. Bristow, Ferguson, Mintz and Rapaport,[29] have measured left ventricular end-diastolic and end-systolic volumes in anesthetized dogs and showed a decrease in these volumes with increases in right atrial pacing rate.

Rushmer's studies[27,28] on conscious dogs emphasize the significant differences in effect on ventricular function between a heart rate increase resulting from atrial or ventricular pacing and the rate increase associated with the exercise response in conscious dogs. He noted that during exercise although heart rate increased markedly, stroke volume was either unchanged or slightly increased. Stroke work increased slightly and peak dP/dt was significantly increased. In contrast atrial pacing routinely caused a decrease in stroke volume and stroke work. Peak dP/dt was only insignificantly increased. The maintenance of stroke volume during exercise was apparently associaed with an increased ejection fraction resulting from increased cardiac sympathetic nerve activity.

Mitchell, Gilmore, and Sarnoff[30] studied the effect of increasing heart rate by right atrial pacing on the relation between mean left atrial pressure and left ventricular end-diastolic pressure. They observed that as the heart rate was increased in progressive steps up to 236 per minute the mean left atrial pressure became progressively higher than left ventricular end-diastolic pressure. When left ventricular function curves were constructed for each atrial

rate the relation between left ventricular end-diastolic pressure and stroke work was unaffected up to heart rates of 158 per minute and at rates of 180 and 200 per minute deviates only at the highest work value examined.

They noted that as pacing rate was increased higher and higher above approximately 139 beats per minute a larger and larger portion of atrial systole occurred prior to opening of the mitral valve, and of course ventricular diastole became shorter and shorter. These observations are not in agreement with those of Berglund, Borst, Duff and Schreiner[31] who found a close correlation between left ventricular end-diastolic pressure and left atrial mean pressure at both high and low heart rates. Finally, these investigators observed that when stroke work was plotted against mean left atrial pressure the function curves showed a shift to the left as heart rate was increased by atrial pacing, however, when these same stroke work values were plotted against left ventricular end-diastolic pressure, and vide infra end-diastolic ventricular volume, no such shift was seen. The implication was clear from these studies that myocardial contractility was not significantly altered by atrial pacing up to rates of approximately 180 per minute. Looking at this another way, one might state that if there were changes in myocardial contractility these changes had little or no effect on their left ventricular function curves. Several years later the studies of Covell, Ross, Sonnenblick, and Braunwald[9] clarified this point. For they showed that while force-velocity curves, simultaneously constructed along with ventricular function curves (stroke work/left ventricular end-diastolic pressure), shifted definitely to the right with increased heart rates (produced by atrial pacing), the left ventricular function curve was unaffected. The changes in myocardial contractility produced by right atrial pacing is admitted to be small, and these observations do imply some insensitivity of the left ventricular function curves to small changes in myocardial norepinephrine concentration and/or contractility.

In 1969, Leighton, Zaron, Robinson and Weissler[32] studied the effect of atrial pacing on hemodynamic data and systolic time intervals in 10 patients with heart disease, eight of whom had "left ventricular dysfunction" and the other two had mitral stenosis. They concluded that "during atrial pacing there was no significant alteration in cardiac output, minute work, left atrial or pulmonary wedge mean pressure, or duration of left ventricular pre-ejection period." They did observe as others have, however, that aortic diastolic pressure rose while left ventricular end-diastolic pressure fell as the pacing rate was increased. They also noted a significant rise in peak dP/dt in three of the patients with the increase in atrial pacing rate. In all these studies a moderate increase in heart rate (an average of 44 beats/min) was used. These authors also were impressed that the fall in left ventricular end-diastolic pressure, and vide infra end-diastolic volume, could not be simply explained by an ineffective atrial contraction, at high heart rates, as claimed by others and supported by their own observation of fusion of the V and A waves seen in the left atrial pressure pulse. Rather they use the fact that mean left atrial pressure was unchanged to support their contention that "the diminished filling volume of the left ventricle must therefore be accounted for by factors other than systolic emptying such as decreased diastolic filling time or mistimed left atrial systole. . . ." Their argument is unacceptable to this reviewer.

Linhart[11] has recently reported his studies in patients where he used atrial pacing to alter left ventricular end-diastolic pressure and stroke work in order to produce what he has termed "pacing" ventricular function curves. He chose to evaluate the merit of using these curves for comparison of patients with normal and abnormal left ventricular function. He showed that the character of the pacing ventricular function curves he obtained with atrial stimulation permitted a clear separation of normal and abnormal responses, and that they were particularly useful in identifying patients with valvular disease (without insufficiency) with an associated decrease in myocardial function. He noted, that as heart rate was increased with atrial pacing there was a decrease in left ventricular end-diastolic pressure in both the normal and abnormal ventricle.

Parker, Khaja and Case[33] have recently

(1971) reported that while the calculated average slope for the ventricular function curve relating stroke volume to left ventricular end-diastolic pressure was steeper in normal subjects than in a group of patients with elevated left ventricular end-diastolic pressure, there was considerable overlap between the two groups. Because of this observation, they felt that "pacing" ventricular function curves had their greatest value in assessing the effects of various interventions (such as drugs) which augment or depress ventricular function in an individual patient. They were not impressed with the adequacy of these curves as a means of discriminating between patients or detecting early signs of ventricular dysfunction.

In 1970 Daggett, Bianco, Powell and Austen[34] showed that both the AS-VS interval and the pattern of ventricular activation importantly influence ventricular function during cardiac pacing. It was noted, however, that when the A-V delay during sequential pacing was adjusted so as to match the AS-VS interval prevailing in the unpaced dog, the hemodynamic state of the ventricle was apparently unaffected by the aberrant nature of ventricular depolarization that occurs during direct ventricular electrical stimulation. They therefore, concluded that with properly timed sequential pacing, ventricular function closely approximates that obtained during a normally propagated wave of depolarization.

Some questions emerge when considering the usefulness of "pacing" ventricular function curves for continued evaluation of ventricular performance in conscious dogs. These are: (1) what effect does left atrial or left ventricular pacing at different rates have on myocardial contractility in conscious dogs; (2) what is the character of the function curves when stroke work is plotted against end-diastolic volume and not end-diastolic pressure; (3) can these curves be made to shift to the left when conscious dogs are infused with either norepinephrine or to the right when the animal has been given a beta blocking dose of propanolol; (4) how reproducible are these effects; (5) is there a clear difference between the hemodynamic effects produced by atrial pacing as compared to left ventricular pacing.

Since we have developed in our laboratory conscious instrumented dogs from which the changes in aortic, left atrial and left ventricular pressures, left ventricular internal diameter, the left atrial and left ventricular electrograms can all be monitored directly, simultaneously and continuously, we felt it appropriate to conduct a systematic study on the effect of atrial and ventricular pacing on ventricular function. The monitored primary variables as listed above when fed thru an A to D converter to our digital computer (HP2116B(16K) and peripherals) provides practically on-line computation of a variety of derived variables for each chosen cardiac cycle. By utilizing this data acquisition and reduction system we have been able to follow the changes occurring in dP/dt, heart rate, total cycle length, end-diastolic, end-systolic and stroke volumes, stroke work and stroke power along with changes in the primary variables and a variety of other derived data for a series of consecutive cycles at different atrial and ventricular pacing rates.

This study presents our findings on the efficacy of using "Pacing" Ventricular Function Curves as one means of evaluating ventricular performance in conscious instrumented dogs.

METHODS

The Conscious Instrumented Dog

Dogs weighing approximately 25 kilograms and of either sex were used in this study. Dogs were obtained in groups of 10 and housed in the Animal Section for at least one month during which time they were improved nutritionally, dewormed and their general health status determined. Those animals found to be in good clinical condition at the end of this period were then operated and instrumented as shown diagramatically in Figure 1. All dogs were instrumented in this way. In some of the animals either an inflatable silastic aortic cuff or a flow probe was placed about the ascending aorta after the latter has been covered with a segment of Dacron.

A considerable period of time has been required to develop our techniques and procedures before finally arriving at the stage of develop-

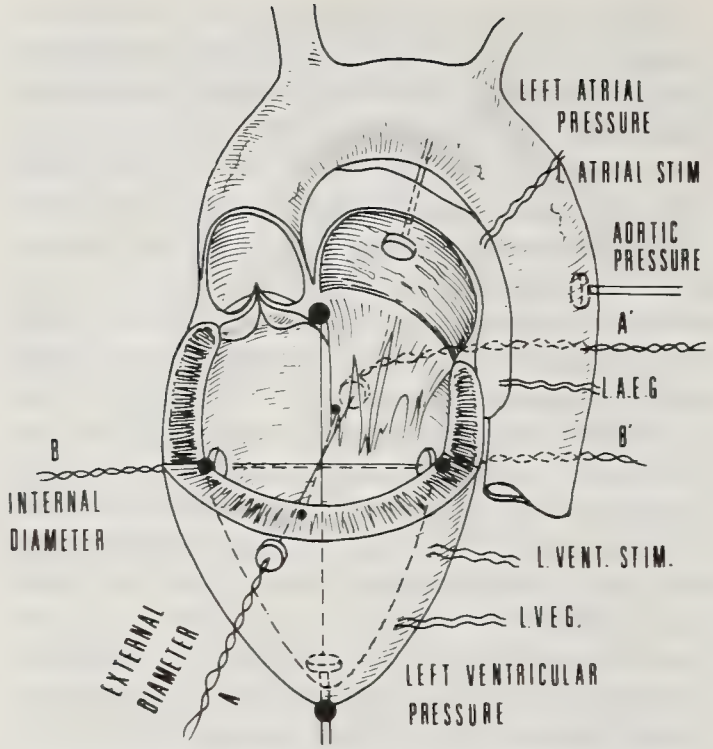


FIGURE 1.—Schematic diagram showing instrumentation of conscious instrumented dogs.

ment of our "instantaneous package", where at present 85% of our instrumented animals survive for at least 5 months in good clinical condition and with all their transducing devices functioning. Of these survivors approximately 50% will continue to be satisfactory for a period of 6 months and 10–15% will last eleven months and perhaps more.

It has been only during the past year that all the current techniques have begun to work to our satisfaction and we have no idea what will be our ultimate survival time with satisfactorily functioning transducing devices.

Our instrumentation package is designed to provide for recording the minimum set of primary variables that will allow for a satisfactory evaluation of left ventricular performance. At present this requires simultaneous recordings of left atrial pressure, left ventricular pressure, aortic pressure, intrathoracic pressure, left ventricular internal diameter, left ventricular external diameter, the left atrial electrogram and the left ventricular electrogram. These can be recorded continuously for periods of time varying with the needs of the investigator.

The conscious instrumented dogs are routinely studied while standing quietly in a modified Pavlov stand. The dogs are trained and are able to comfortably remain in the stand for as long as 12 hours, without becoming disturbed. "Hook up" time is approximately 30 minutes and either semicontinuous or continuous monitoring is then possible. Each dog is usually monitored at least once a week and in some cases three times a week depending on the experimental objectives.

Instrumentation for Measuring Pressures

Implantable pressure transducers and small polyvinyl tubes are used for measuring pressures. The implantable pressure cells are made by Konigsberg Instruments Company.* The cells usually used are 6.5 mm in diameter. These implantable cells, with their lead wires, have remained in two dogs for as long as eleven months without failure.

The implantable cells are surgically inserted in the locations as diagrammed in Figure 1. The lead wires from the cells, diameter gages, and electrode wires are exteriorized in the middle of the back of the dog's neck. The neck of the dog is wrapped once or twice weekly using gauze and "kling bandage" and a good quality adhesive tape. Each dog is checked daily to insure protection of these wires.

Calibration of the implanted pressure cells is accomplished in the following way at present. A small bore polyvinyl tube is inserted into the ventricle, aorta and atrium along side the pressure cell. The tubing and the technique for its use in implantation has been described by Barger et al.[36] These tubes are flushed daily for the first two weeks with a weak Heparin solution and then once or twice weekly. The tubes are brought out through the left chest wall at the mid-axillary line and at heart level. Pressures are measured again at atmospheric pressure using Statham P23DB strain gages, simultaneously with the pressures recorded from the pressure cells. This is done at the start and end of a recording run to calibrate the pressure cells. The recording system is very stable and permits periods of recordings up to 12 hours without drift. Appropriate adjustments in the

* Konigsberg Instruments Company, 2010 Foothill Boulevard, Pasadena, California

calibrations are made for large changes in barometric pressure when this occurs. Similarly intrathoracic pressure is calibrated when desired. In the interim between comparison calibrations, D.C. voltage levels representative of the established calibration positions (high and low values) are used to "tell" the computer where 0 and 50 mm of mercury is or some similar set of values. The amplifiers and recording system are held fixed at one sensitivity and balance position during these times. In addition, every precaution is taken to have accurately calibrated recordings.

Measurement of Diameter (Inductance Micrometry)

Pairs of induction coils are used to measure diameters. One pair is placed internally across the diameter on the plane of the minor semiaxis of the left ventricle, and one pair is fixed to the external surface of the left ventricle in the same plane. Both of these are diagrammed in Figure 1.

Inductance micrometry involves exciting one coil of each pair with an oscillating sine wave current at constant voltage and amperage and monitoring the amplified, rectified, and demodulated induced EMF from the opposite receiving coil of each pair. A linearized output is achieved through a specially designed circuit (Perry, Hinds, Hawthorne: A linearizing inductance micrometer for monitoring left ventricular internal diameter). This permits calibration of the coils in terms of the amount of separation in centimeters versus voltage output. The recorded variations in D.C. voltage with changes in ventricular diameter is a linear and inverse function of separation of the coils over a range of 2.5 to 6 mm separation.

Electrograms and Pacing Wires

Pairs of teflon coated multistranded stainless steel wires are used for electrodes and pacing wires. Bipolar electrograms are recorded from the left atrium and left ventricle.

Lead Beads

Shown in Figure 1 is the placement of pairs of lead beads of different sizes. These are inserted to a subendocardial position at the time of surgery. We use these opaque markers to study change in left ventricular length and diameter by biplane cineradiography.

Pre and Post Operative Care

All dogs are kept continuously under close medical supervision and appropriate antiarrhythmic, antibotic, nutritional and other indicated therapy is continuously available to them. The dogs chosen for study are kept in excellent clinical condition. We do not use animals which have high temperatures, diarrhea, worms, pleural fluid, pericardial effusion and the like. No dog is seriously studied until at least two weeks post operative. All animals are given atropine, xylocaine, and a broad spectrum antibiotic prophylactically during the first 48 hours after surgery. Antibiotics are continued until body temperature has returned to normal. We use the dog's instrumentation to observe the condition of these animals, through continuous monitoring, until they recover from anesthesia for the first 5 hours after surgery and for 12 days post operative. Since all the dogs collect fluid during this period, the chest is drained of all collected fluid. After the first 12 post operative days no further fluid sems to collect in the chest of most dogs. Those that still have effusion at this time usually do not recover satisfactorily. The chest drainage tube is removed at this time. Chest x-rays are made at infrequent intervals in all dogs.

Data Acquisition and Reduction

With the transducers in place, as described above, their voltage outputs are cabled to the appropriate carrier, D.C. coupling, high and low gain amplifiers. The amplified and conditioned signals are then distributed, as required, by patch panel connections to (a) recording system for permanent graphic chart of analog signals; (b) to a multichannel scope for monitoring and display; (c) to an analog magnetic tape recorder for storage of all data signals; (d) to an A to D converter for input to the computer. In this mode, data can be sampled on line, as well as retrieved from magnetic tape for study and selection of portions for detailed processing by the computer. The data is proc-

essed by means of a Hewlett-Packard 2116B digital computer, with 16K word of main memory (16 bit words) and two direct memory access channels allowing data transmission rates up to 625 KHz by interleaving the channels. Peripheral devices include a multiplaced 16 channel, 14 bit resolution A to D converter capable of throughput rates of 18 KHz, two moving head disc drives each with a fixed disc a removable cartridge providing a peripheral storage capability of 2.6 million words, accessible at a transfer rate of 45 KHz, a 7 channel digital magnetic tape unit (200 bits/in., 30 in./sec.) ; a high speed printer (300 lines/min.) ; and a Tektronix 4002A graphic terminal display unit. The hardware system is supervised by a HP-DOSM operating system with special input/output routines to facilitate continuous sampling and storage of large blocks of data (up to 500,000 words).

The first derivative of the left ventricular intracavitary pressure (dP/dt) was obtained by pacing the amplified signal from a pressure cell and feeding it into a Biotronex laboratory active differentiator, (Model 621) where the phase angle was 90° and the center frequency (fo) was set such that the corner frequency was 150 Hz. Amplitude was estimated through the active differentiation of a triangular wave which was adjusted so that the peak to peak amplitude was equivalent to 160 mm Hg and the frequency was varied from 4 to 16 Hz. The resultant square wave at each step served to calibrate the output of the differentiator.

The mean velocity of circumferential fiber shortening (mean V_{CF} (EJ)) during ejection was defined as the extent of shortening of the left ventricular internal circumference, in the midplane normal to the long axis, during the period between end isovolumic contraction and end ejection, divided by the duration of ejection. These points in the cardiac cycle were identified by an algorithm using the computer.

In previous studies we have made measurements of the simultaneous changes in base to apex, length, external circumference and wall thickness in the plane of the minor axis. These measurements have also been made along with estimation of left ventricular length of the major axis using biplane cinefluorography. From these studies we have observed that the shape of the left ventricle can most closely be approximated using geometry of an ellipsoid of revolution. In addition it has been observed that the change in internal axis ratio during ejection is very small. The volume of an ellipsoid of revolution is given by the expression:

$$V = 4/3\,\pi\,R_i^3\,B \qquad (1)$$

V = volume where R = length of ½ the minor axis; B = axis ratio.

In these studies R was measured directly and the axis ratio was established at end diastole and end systole for each dog. The mean of these two values was then used in equation (1) for estimation of internal volume during the phase of ejection.

Left ventricular stroke work for each peak was computed through integration of the left ventricular pressure times the change in intracavitary volume throughout the phase of ejection. The expression used for computation was:

$$SW = 1.36 \times 10^{-2} \sum_{i=1}^{N} \frac{P_i + P_i + 1}{2} (V_i - V_i + 1) \qquad (2)$$

where N = total number of samples taken by computer during the phase of ejection. The estimation of left ventricular endocardial wall force is based on the assumption that in a plane normal to the long axis, at the middle of the left ventricle, the rim of muscle is circular. Then the force acting on the endocardial surface at any given instant is a function of the intracavitary pressure and the internal radius and can be computed from the expression:

$$\text{Wall force} = 1.36 \times \text{LVP} \times \pi\,R_i^2 \qquad (3)$$

The average wall force during ejection is then equal to the sum of forces acting during ejection divided by the duration of ejection. In this study wall force was estimated at 5 msec intervals throughout the cardiac cycle.

A Grass stimulator (Model S8) was used to stimulate the atrium and ventricle. The fre-

quencies used were 2.0, 2.5, 3.0, 3.5, 4.0; the duration was 3 msec, and voltage 3–5. The frequencies were used only as a reference for the relative level of stimulation. Heart rate and cycle length was calculated by the computer on the basis of definition of the cardiac cycle.

RESULTS

A typical recording from a conscious instrumented dog, 45 days after surgery for implantation of transducing devices, is shown in Figure 2. This recording was taken during the control period for one experiment where the dog is standing quietly in a stand as described above. Of interest is the characteristic fidelity of the recordings of the primary variables being monitored. Such detail is seen for example on the left atrial pressure pulse tracing where the distinct components of the wave form is easily discerned. All phases of the cardiac cycle can be readily identified from these data for specific analysis. In addition, the low heart rate and marked sinus arrhythmia is a characteristic finding of the conscious dog in good health in our laboratory. These recordings are stable and allow performance of a variety of experiments.

Pacing and Left Ventricular Size

The Atrium

The typical response of one dog to different frequencies of atrial pacing is shown in Figures 3 and 4. Left ventricular end-diastolic internal diameter consistenly decreased with successive increases in frequency of stimulation of the atrium. However there was no significant change in end-systolic diameter. Figure 4 is a faster recording which shows some of the detail attending atrial pacing. In addition to the changes seen in heart size—at high atrial pacing rates[4] there is demonstrated in left atrial pressure pulse tracing the presence of fusion of "V" and "A" waves. Figure 5 shows the responses during sequential pacing of the left atrium and ventricle. During these studies, the interval between atrial systole and ventricular systole (AS-VS interval) was 100 msec. Sequential pacing of the heart resulted in a persistent decrease in end-diastolic and end-systolic diameters. As shown in Figure 6, ventricular pacing resulted in a decrease in end-diastolic internal diameter with little change at end-systole. Tables I and II show the changes in end-diastolic volume (EDV) observed during atrial and ventricular pacing.

Pacing and Myocardial Contractility

In Figures 3, 4, 5, are shown the changes in peak dP/dt and peak d lnP/dt observed during atrial and sequential pacing. It is to be noted that although the mean LVP during ejection is significantly increased over the control level with atrial pacing, the mean ventricular pressure is not significantly different from one pacing rate to the other. With each increase in frequency of stimulation there is an increase in peak dP/dt and peak d lnP/dt. In addition, as shown in Table II, the average wall force during ejection is decreased with each frequency of stimulation while there is a persistent increase in peak dP/dt and mean V_{CF} above the control values for these variables. Similar observations are made during periods of sequential pacing. In contrast, pacing of the left ventricle resulted in a decrease in mean LVP, with no significant difference from one pacing rate to the other. There is also significant difference in the average wall force during ejection with each increment in frequency of stimulation of the ventricle. Further, peak dP/dt and mean V_{CF} tend to remain constant.

Pacing and the Stroke Work (SW) End-Diastolic Volume (EDV) Relation

In these studies we have found it difficult to always capture and pace the atrium. The typical effects of atrial pacing in one dog is shown in Table II. A facsimile of the computer plot of the stroke work EDV relation is shown in Figure 7 along with its computed linear regression equation labeled (1). There is some scatter of the data around the regression line. Figure 8 shows the regression line for atrial pacing (1) along with the data for ventricular pacing (2) in the same dog. It can be seen that there is a linear relation between stroke work and end-diastolic volume. The profile of the curve unlike that curvelinear relation that is usually seen for the

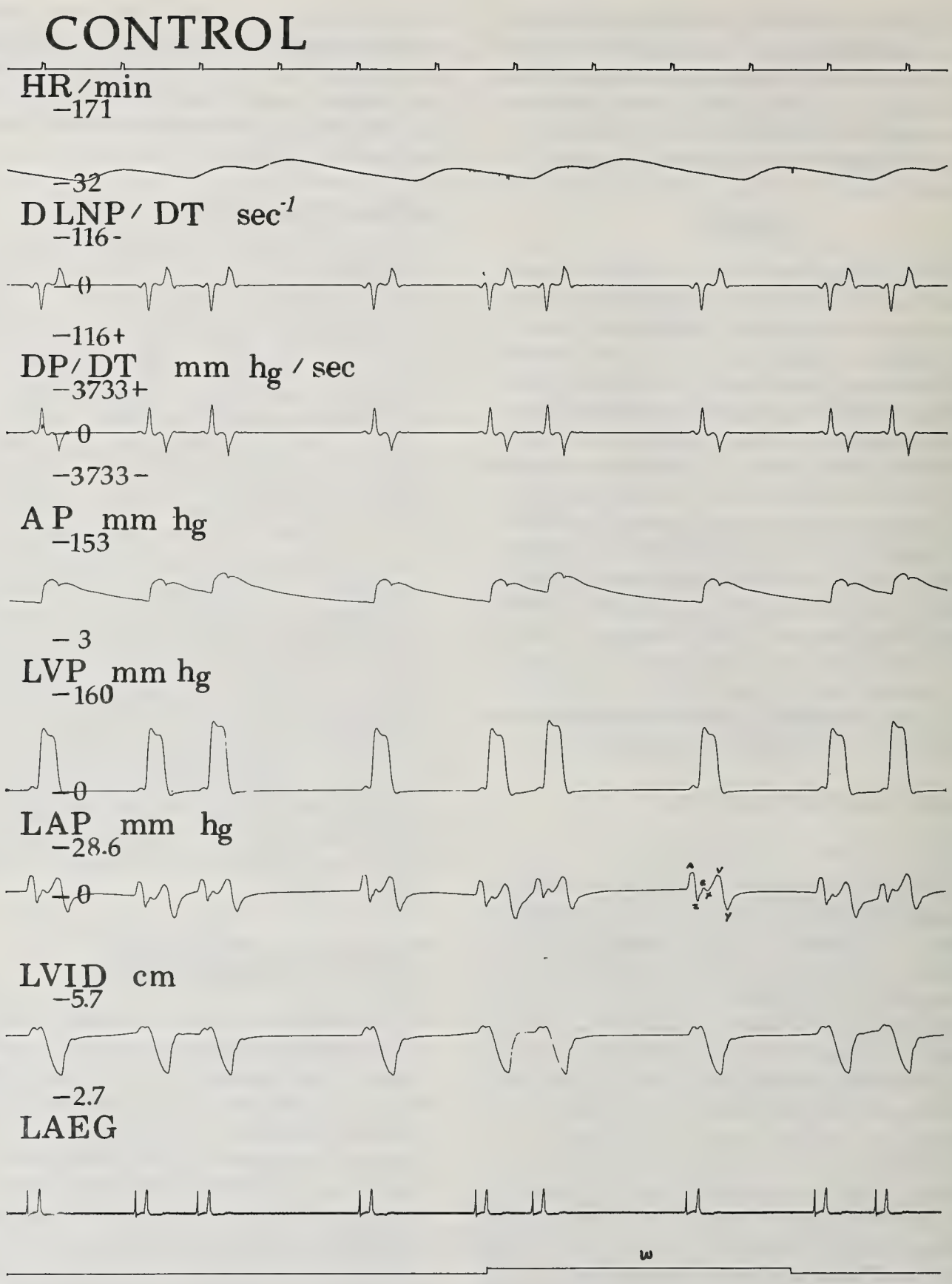


FIGURE 2.—Typical recording from a conscious instrumented dog 45 days after surgery for implanting transducing devices. HR = heart rate, d lnP/dt = the first derivative of the natural logarithm of the left ventricular pressure; dP/dt = first derivative of the left ventricular pressure; AP = central aortic pressure; LVP = left ventricular pressure; LAP = left atrial pressure; LVID = left ventricular internal diameter; LAEG = left atrial electrogram.

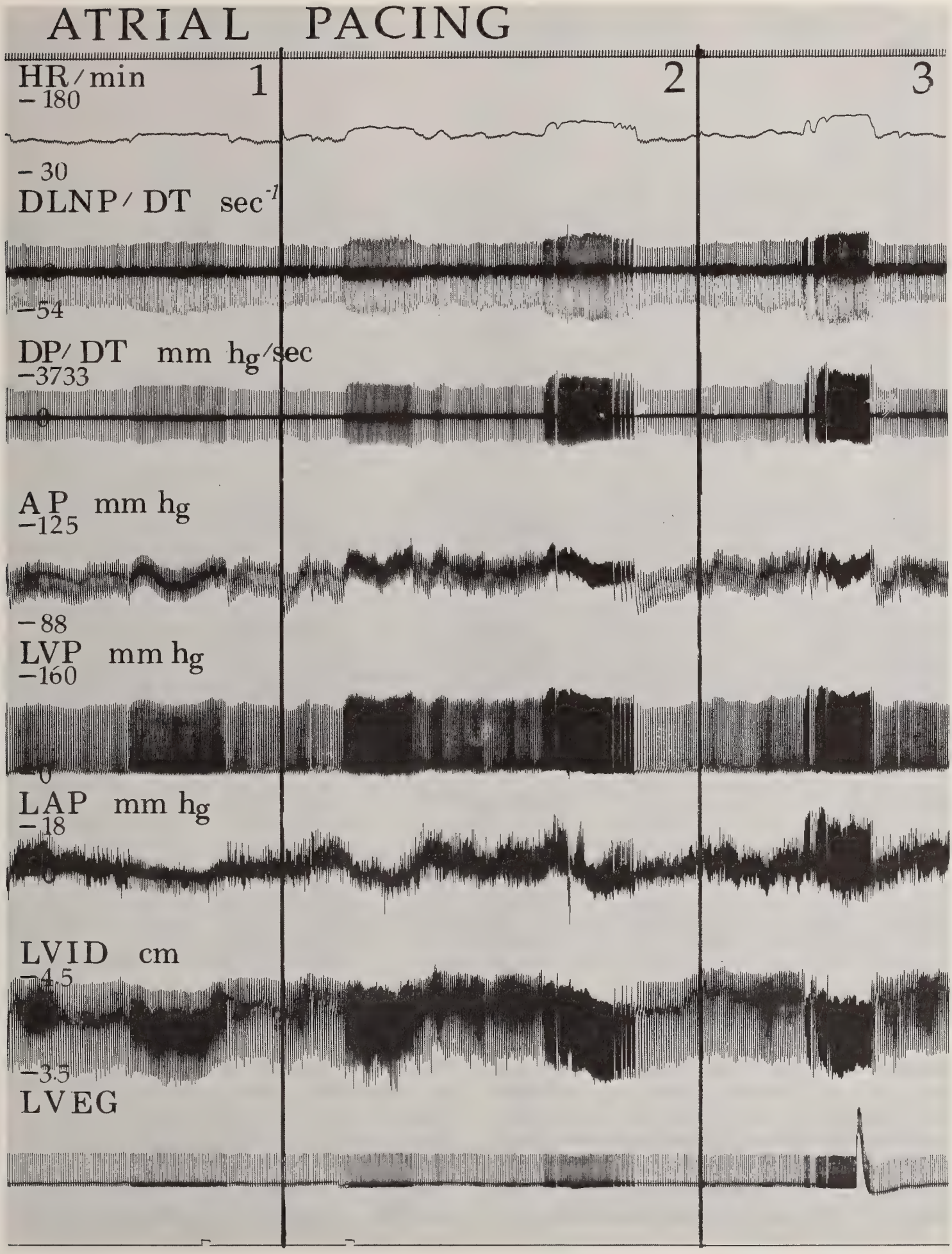


FIGURE 3.—Effects of atrial pacing, using bipolar stimulation, at different frequencies. LVEG = left ventricular electrogram. Other labels are the same as used in Figure 2.

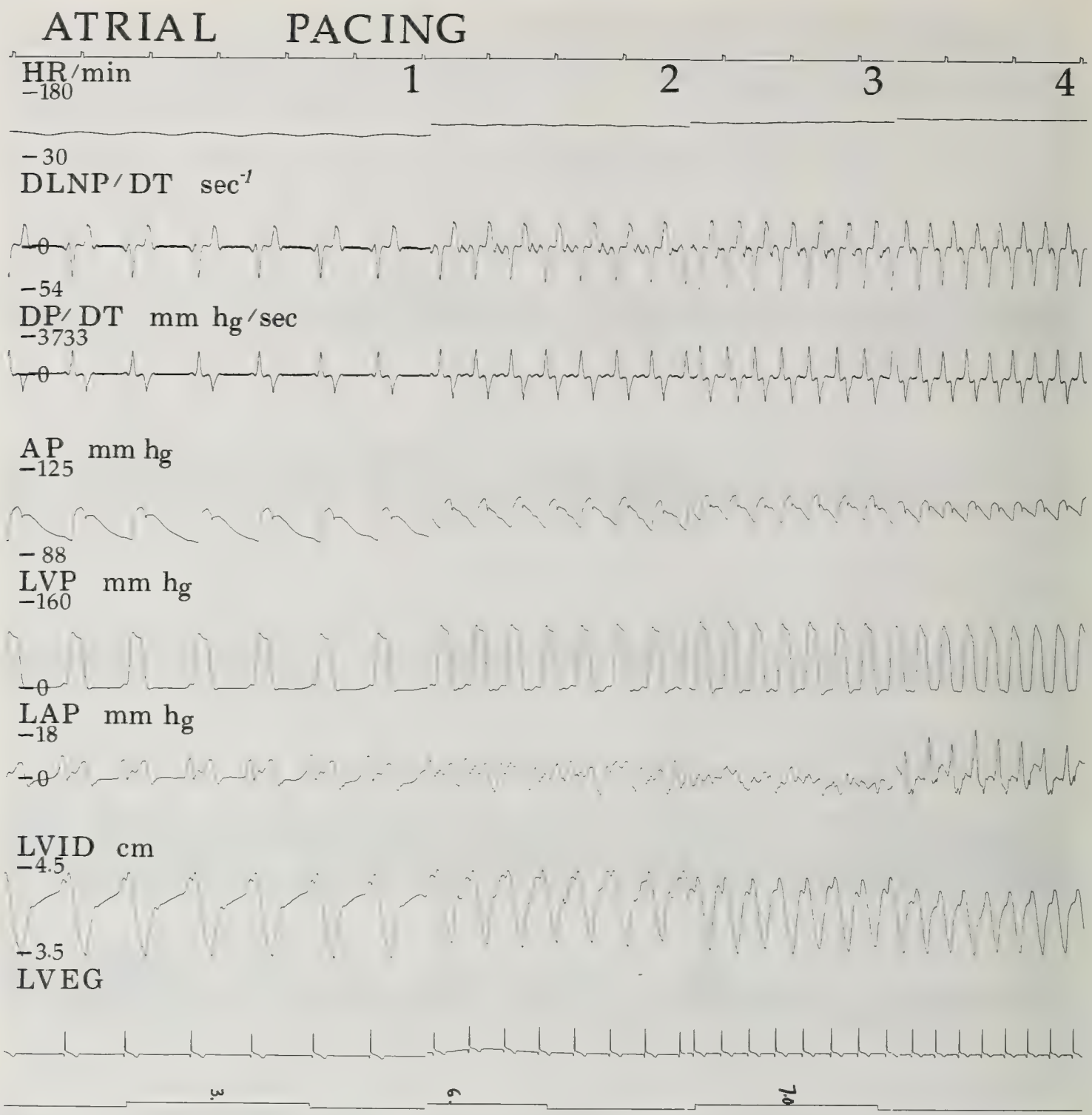


FIGURE 4.—Same as Figure 3 at higher paper speed. Note the high V waves on the left atrial pressure pulse at the high pacing rate (panel 4).

ventricular function curve. Further there is far less scatter about the regression line. These data show that for any given end-diastolic volume, atrial pacing results in more stroke work than ventricular pacing. The intersection of lines (1) and (2) is for control data for each period of pacing. The finding of a linear relation between SW and EDV during ventricular pacing and the ease with which control of the ventricle was obtained led us to investigate the feasibility of using ventricular pacing in the genesis of ventricular function curves.

In Figure 9 is shown the regression equation for atrial pacing (1) and ventricular pacing

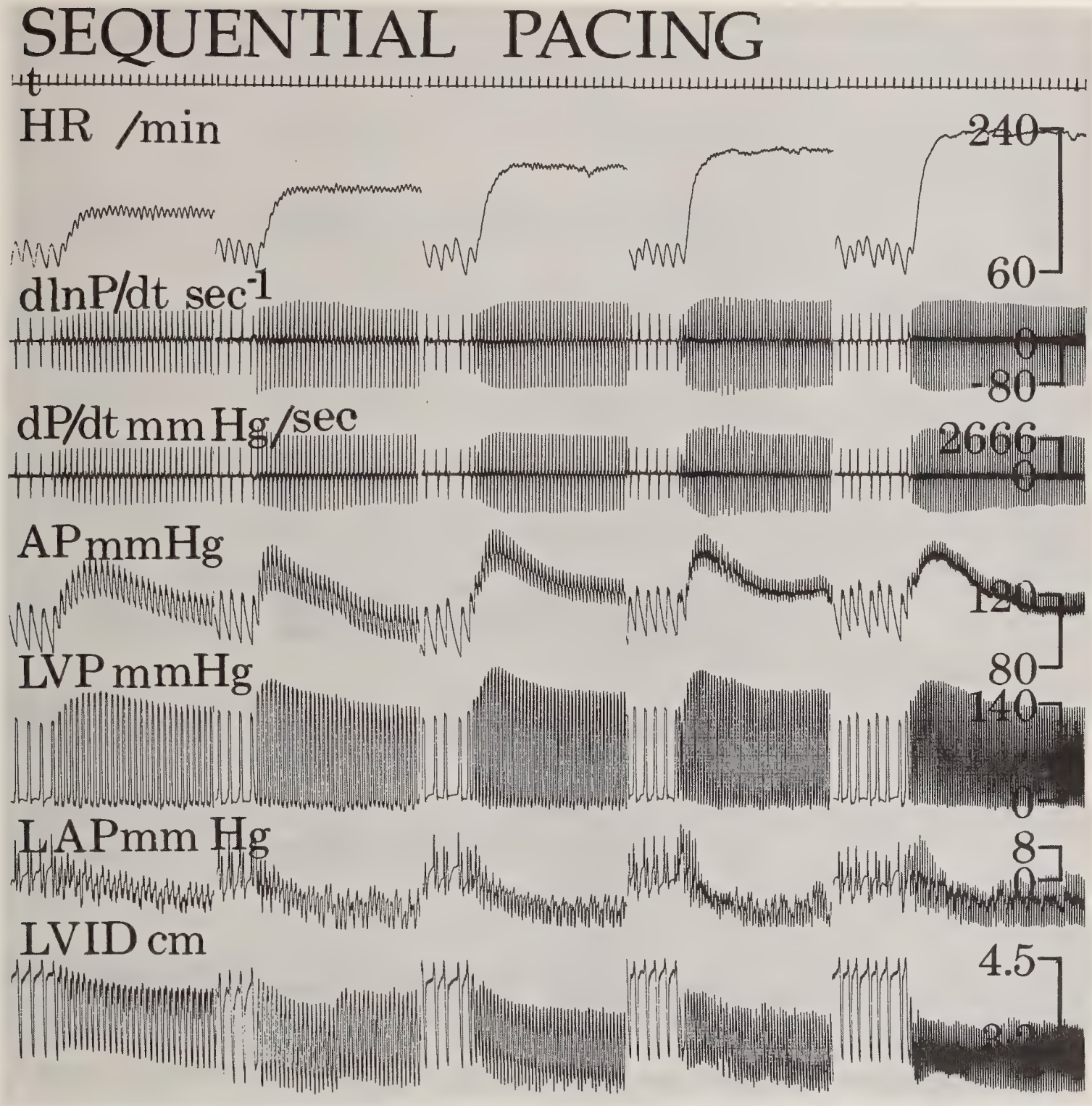


FIGURE 5.—Effects of sequential pacing using and atrial stimulus to ventricular stimulus interval of 100 milliseconds. Labels are the same as used in Figure 2.

(2). In addition, the data points obtained during ventricular pacing while norepinephrine was being infused at a rate of 0.4 mg/Kg/mm. Comparison between lines (2) and (3) shows that there is shift of the curve upward and to the left which is reminiscent of the classical ventricular function curve. Under the influence of norepinephrine there is a greater stroke work for any given end-diastolic volume.

Figure 10 shows the regression lines (1), (2), and (3), as described along with data obtained with ventricular pacing and propranolol (0.5 mg/Kg). Comparison of line (2) and line (4) shows that for any given end-diastolic vol-

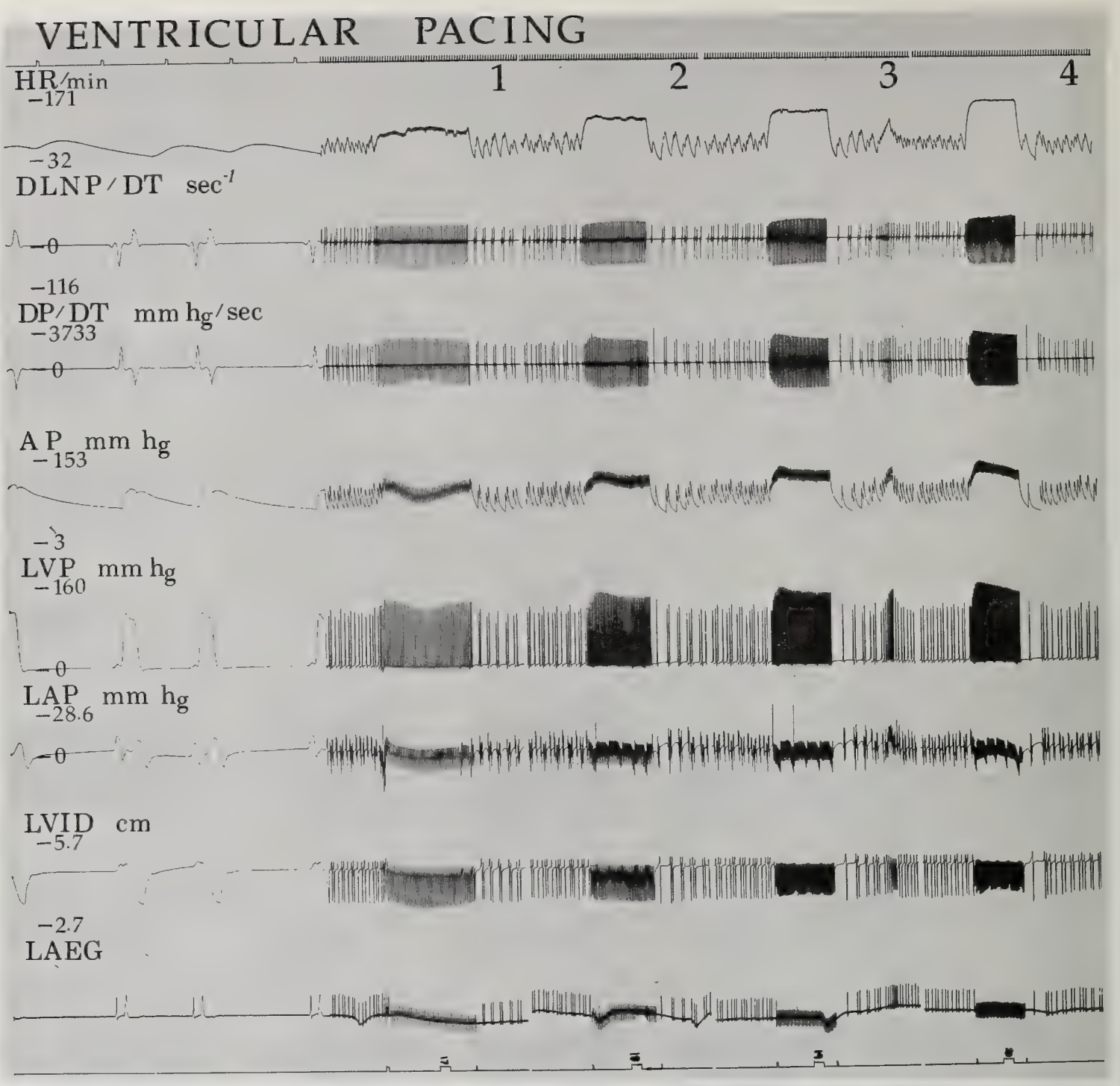


FIGURE 6.—Effect of ventricular, bipolar stimulation, at increasing pacing rates. Labels are same as in Figure 2.

ume there is reduction in the amount of stroke work which the ventricle generated under the influence of propranolol. It is to be noted that the usual result seen with propranolol was no change in SW/EDV relation from that of the control curve. Table I shows the data on which the computer plots for ventricular pacing are based. In addition, these data show that with norepinephrine infusion there was a significant increase in peak dP/dt and an increase in mean V_{CF} and average wall force. Propranolol injection caused a marked decrease in peak dP/dt, and a decrease during ejection in mean V_{CF} and average wall force at the endocardial surface of the left ventricle.

SUMMARY

It is well known that increasing the rate of ventricular contraction by artificial stimulation

TABLE I.—*Changes with Ventricular Pacing (Dog # 132 11th P.O. Day)**

N	TCL (msec)	LVEDP (mm Hg)	EDV (ml)	SW (gm-m)	Peak dP/dt (mm Hg/sec)	Mean (EJ) LVP (mm Hg)	Aver. WF(EJ) (gms)	Mean (EJ) VCF (Cm/sec)
				Control Study				
14	510 ± 34	15 ± 5	52 ± 3	50 ± 4	3860 ± 157	140 ± 4	1832 ± 89	30.4 ± 2
14	488 ± 53	9 ± 4	47 ± 5	44 ± 6	3896 ± 300	138 ± 6	1786 ± 124	27.6 ± 3
31	**368 ± 12	6 ± 2	39 ± 2	27 ± 3	3766 ± 136	116 ± 5	1268 ± 95	33.4 ± 2
25	**314 ± 3	5 ± 3	37 ± 1	24 ± 1	3638 ± 82	120 ± 2	1303 ± 34	30.8 ± 1
31	**269 ± 4	5 ± 4	33 ± 2	23 ± 3	4102 ± 363	128 ± 9	1370 ± 137	29.8 ± 2
				Norepinephrine (0.4 mgms/Kg/min)				
10	714 ± 79	16 ± 2	57 ± 3	67 ± 7	6452 ± 277	161 ± 8	1924 ± 122	42.9 ± 2
13	628 ± 125	15 ± 3	58 ± 2	70 ± 10	6826 ± 257	164 ± 9	2020 ± 222	43.6 ± 3
22	**366 ± 3	17 ± 4	58 ± 3	68 ± 6	8698 ± 242	214 ± 5	2885 ± 129	39.2 ± 3
30	**311 ± 7	11 ± 3	51 ± 2	61 ± 4	9026 ± 324	189 ± 5	2275 ± 74	46.8 ± 2
30	**265 ± 3	6 ± 4	42 ± 3	49 ± 3	8450 ± 362	177 ± 4	1929 ± 75	46.5 ± 3
				Propranolol (0.5 mgms/Kg)				
12	560 ± 19	14 ± 2	60 ± 1	50 ± 3	3159 ± 61	134 ± 3	2050 ± 80	26.1 ± 1
12	533 ± 16	14 ± 2	56 ± 2	48 ± 3	3178 ± 49	132 ± 4	1958 ± 83	25.9 ± 1
20	**364 ± 2	11 ± 2	52 ± 1	31 ± 2	3110 ± 79	126 ± 2	1741 ± 44	29.2 ± 1
30	**309 ± 3	9 ± 3	51 ± 1	28 ± 3	3143 ± 109	122 ± 4	1713 ± 78	27.4 ± 2
30	**268 ± 3	11 ± 2	48 ± 3	26 ± 6	3338 ± 221	127 ± 4	1788 ± 86	24.7 ± 3

* Shown above are the effects of ventricular pacing of a conscious dog 11 days after surgery for instrumentation. Consecutive cardiac cycles were analyzed for periods of sinus rhythm and while the left ventricle was paced at various rates. The observations were made during a control period, during the steady state while infusing norepinephrine, and after subsequent intravenous injections of propranolol. The results are typical for the group of dogs studied. N = the total number of consecutive cardiac cycles analyzed in each sample; TCL = total cycle length; LVEDP = left ventricular end-diastolic pressure; EDV = end-diastolic volume; SW = stroke work; mean LVP (EJ) = average left ventricular pressure during ejection; Aver. WF(EJ) = average wall force during ejection; mean V_{CF} = average velocity of internal circumferential shortening rate during ejection. All values are mean ± SD.

** Ventricle paced.

decreases ventricular volume in conscious dogs. This observation was fully investigated by Rushmer and associates[28]. Since left ventricular pacing at rates from 2 to 3.5 Hz causes stepwise decreases in ventricular volume and stroke work without significantly altering myocardial contractility, it appears that an acceptable ventricular function curve can be constructed from these data. The function curve shifts upward and to the left under conditions of increased myocardial contractility. A shift downward and to the right is produced with depression of ventricular function. These shifts are consistent with and as predicted by the classical studies of Sarnoff and his associates. It therefore appears that left ventricular pacing in conscious instrumented dogs is a reliable means of producing a spectrum of end-diastolic volumes for acquiring the primary information necessary to study the shifts in the stroke work/end-diastolic volume relation with changes in ventricular function brought on by various interventions. Analysis of function curves produced by either atrial or sequential pacing as compared to those produced by left ventricular pacing shows that with atrial or sequential pacing for any given end-diastolic volume the stroke work produced is greater than with ventricular pacing. Our studies suggest that this difference is due largely to the increased contractility at each pacing rate that occurs with atrial or sequential pacing. No evidence is provided to determine whether or not the aberrant ventricular my-

TABLE II.—*A Comparison of Atrial and Ventricular Pacing (Dog # 132 11th P.O. Day)*

N	TCL (msec)	Mean* LVP (mm Hg)	Aver. WF* (gm-m)	Peak dP/dt (mm Hg/sec)	Mean** VCF (Cm/sec)
		Left Atrial Pacing (P) (SW = −9.65 + EDV × 1.13)			
9	748 ± 160	129 ± 6	1703 ± 95	3792 ± 126	28.3 ± 1
12	753 ± 154	131 ± 6	1734 ± 108	3819 ± 106	28.7 ± 1
22	P376 ± 8	141 ± 8	1611 ± 67	5258 ± 321	33.7 ± 3
27	P338 ± 51	139 ± 6	1539 ± 89	4469 ± 522	33.0 ± 5
30	P288 ± 43	125 ± 6	1261 ± 59	4294 ± 605	33.0 ± 6
		Left Ventricular Pacing (V) (SW = −31.5 + EDV × 1.55)			
14	510 ± 34	140 ± 4	1832 ± 89	3860 ± 157	30.4 ± 2
14	448 ± 53	138 ± 6	1786 ± 124	3896 ± 300	27.6 ± 3
21	V368 ± 12	116 ± 5	1268 ± 95	3766 ± 136	33.4 ± 2
25	V314 ± 3	120 ± 2	1303 ± 34	3638 ± 82	30.8 ± 1
31	V269 ± 4	128 ± 9	1370 ± 138	4102 ± 364	29.8 ± 2

* = During ejection

** = Endocardial surface

All values are mean ± SD

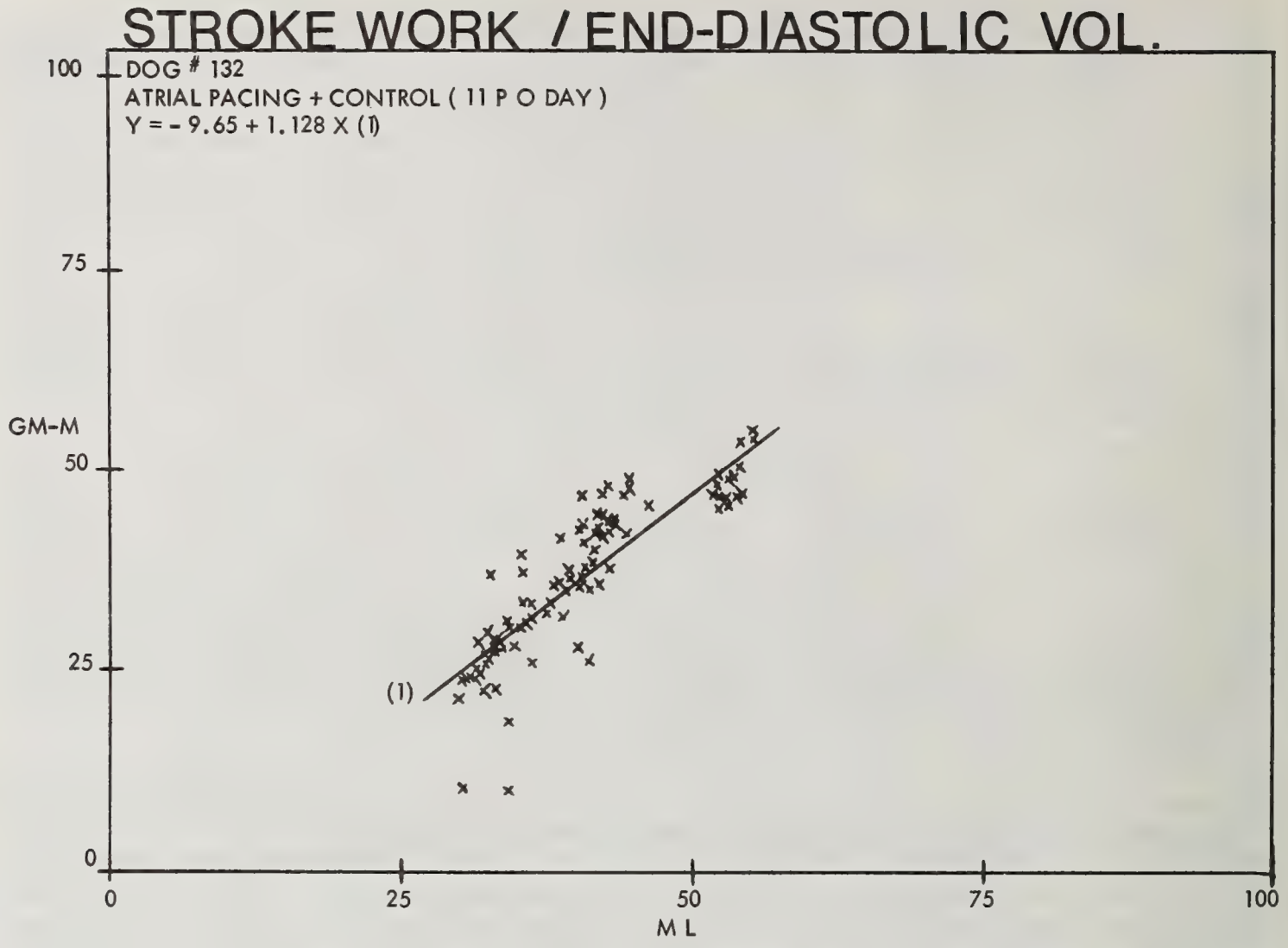


FIGURE 7.—A copy of the computer plot of the stroke work/end-diastolic volume relationship obtained with atrial pacing. Y = stroke work and X = end-diastolic volume. The regression line was plotted from the equation (1). The data upon which this plot is based is given in Table II.

ocardial stimulation produced by ventricular pacing causes some alteration in the pattern of ventricular contraction. However, we found that the "pacing" left ventricular function curves produced by ventricular pacing were reproducible from day to day in the same animal.

REFERENCES

1. FRANK, O. Zur Dynamik des Herzmuskels. Z. Biol. 32:370, 1895.
2. STARLING, E. H. *The linacre lecture on the law of the heart.* Cambridge, 1915 London, Longmans, Green & Co., 1918.
3. SARNOFF, S. J., and BERGLUND, E. Ventricular function: 1. Starling's law of the heart studied by means of simultaneous right and left ventricular function curves in the dog. Circulation 9:706, 1954.
4. SARNOFF, S. J., and MITCHELL, J. H. The control of the function of the heart. In *Handbook of Physiology,* Section 2: Circulation, edited by W. F. Hamilton and Philip Dow. Washington, D. C. Amer. Physiol. Soc., vol. 1, chap. 15, p. 489, 1962.
5. SARNOFF, S. J. Myocardial contractility as described by ventricular function curves. Observations on Starling's law of the heart. Physiol. Rev. 35:107, 1955.
6. SONNENBLICK, E. H. Force-velocity relations in mammalian heart muscle. Amer. J. Physiol. 202:931, 1962.
7. SONNENBLICK, E. H. Implications of muscle mechanics in the heart. Federation Proc. 21:975, 1962.
8. SONNENBLICK, E. H. Instantaneous force-velocity-

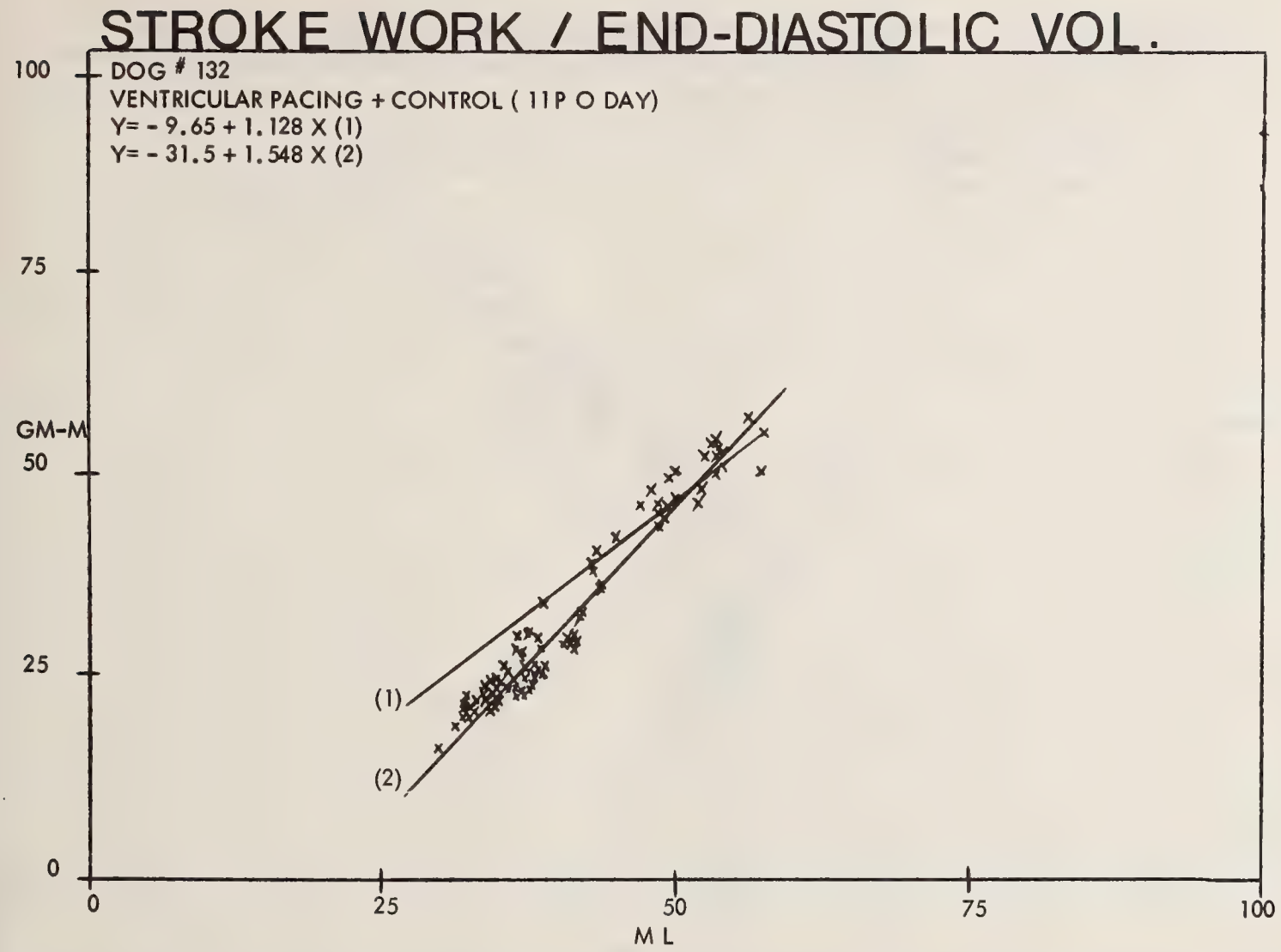


FIGURE 8.—A comparison between the regression lines for atrial (1) and ventricular (2) pacing. The individual points were obtained from the individual cardiac cycles analyzed at different ventricular pacing rates and during a control period. The data upon which this plot is based is shown in Tables I and II.

length determinants in the contraction of heart muscle. Circulation Res. 16:441, 1965.

9. COVELL, J. W., ROSS, JR., J., SONNENBLICK, E. H., and BRAUNWALD, E. Comparison of the force-velocity relation and the ventricular function curves as measures of the contractile state of the intact heart. Circulation Res. 19, 264, 1966.
10. DESANCTIS, R. W. Diagnostic and therapeutic uses of atrial pacing. Circulation 43:748, 1971.
11. LINHART, J. W. Pacing-induced changes in stroke volume in the evaluation of myocardial function. Circulation 43:253, 1971.
12. ROSS, JR., J., and BRAUNWALD, E. Study of left ventricular function in man by increasing resistance to ventricular ejection with angiotensin. Circulation 29:739, 1964.
13. FINNEY, JR., J. O. Hemodynamic alterations in left ventricular pacing. Amer. J. Physiol. 208:275, 1965.
14. KLOTZ, D. H., LISTER, J. W., JOMAIN, S. L., HOFFMAN, B. F., and STUCKEY, J. H. Implantation sites of pacemakers after right ventriculotomy and complete heart block. J. Amer. Med. Assoc. 186:929, 1963.
15. STARZL, T. E., GAERTNER, and WEBB, JR., R. C. The effects of repetitive electric cardiac stimulation in dogs with normal hearts, complete heart block and experimental cardiac arrest. Circulation 11:952, 1955.
16. WILLIAM-OLSSON, G., and ANDERSON, M. N. The effect of pacemaker electrode site on cardiac output. J. Thoracic Cardiovascular Surg. 45:618, 1963.
17. BENCHIMOL, A., and LIGGETT, M. S. Cardiac hemodynamics during stimulation of the right atrium, right ventricle, and left ventricle in normal and abnormal hearts. Circulation 33:933, 1966.
18. FLETCHER, F. W., THEILEN, E. O., LAWRENCE, M. S., and EVANS, J. W. Effect of pacemaker location

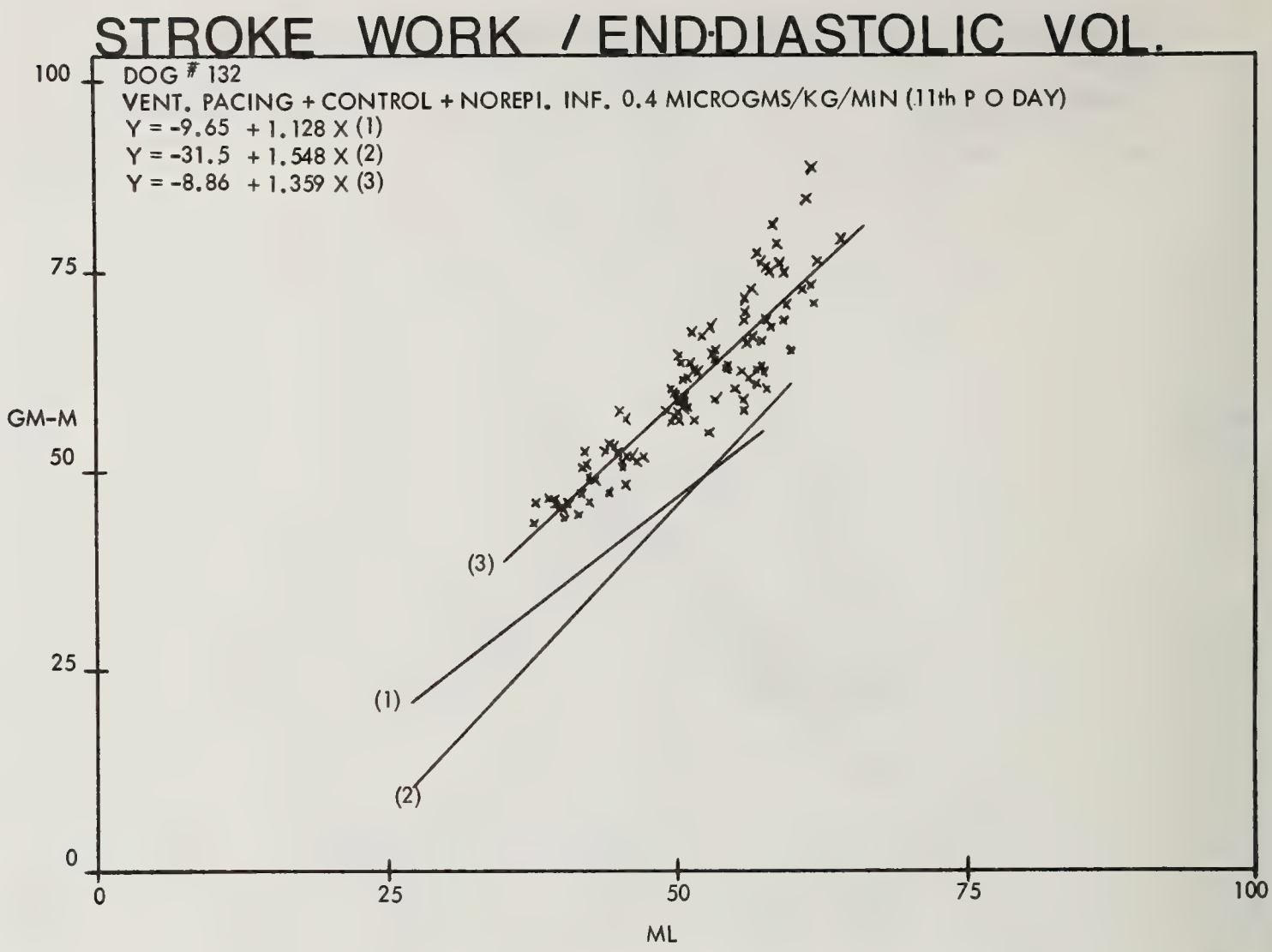


FIGURE 9.—Shows the stroke work/end-diastolic volume relationship during the steady state while infusing norepinephrine (3). The regression lines from Figures 7 and 8 are also shown.

on cardiac function in complete A-V heart block. Amer. J. Physiol. 205:1232, 1963.

19. STEIN, E., DAMATO, A. N., KOSOWSKY, B. D., LAU, S. H., and LISTER, J. W. The relation of heart rate to cardiovascular dynamics. Circulation 33:925, 1966.
20. GILMORE, J. P., SARNOFF, S. J., MITCHELL, J. H., and LINDEN, R. J. Synchronicity of ventricular contraction: Observations comparing hemodynamic effects of atrial and ventricular pacing. Brit. Heart J. 25:299, 1963.
21. GLICK, G., WILLIAMS, JR., J. F., HARRISON, D. G., MORROW, A. G., and BRAUNWALD, E. Cardiac dimensions in intact unanesthetizd man. VI. Effects of changes in heart rate. J. Appl. Physiol. 21:947, 1966.
22. HENDERSON, Y. The volume of the ventricles of the mammalian heart, and the significance of this curve in respect to the mechanics of the heart beat and the filling of the ventricles. Amer. J. Physiol. 16:325, 1906.
23. PATTERSON, S. W., PIPER, H., and STARLING, E. H. The regulation of the heart beat. J. Physiol., London 48:465, 1914.
24. WIGGERS, C. J., and KATZ, L. N. The contour of the ventricular volume curves under different conditions. Amer. J. Physiol. 58:439, 1922.
25. CHAPMAN, C. B. On the nature of cardiac control. Ann. Internal Med. 53:1272, 1960.
26. MILLER, D. E., GLEASON, W. L., WHALEN, R. E., MORRIS, JR., J. J., and MCINTOSH, H. D. Effect of ventricular rate on cardiac output in the dog with complete heart block. Circulation Res. 10:658, 1962.
27. RUSHMER, R. F. Constancy of stroke volume in ventricular response to exertion. Amer. J. Physiol. 196:745, 1959.
28. RUSHMER, R. F., and SMITH, JR., O. A. Cardiac control. Physiol. Rev. 39:41, 1959.
29. BRISTOW, D. J., FERGUSON, R. E., MINTZ, F., and ventricular volume in dogs. J. Clin. Invest. 42:649,

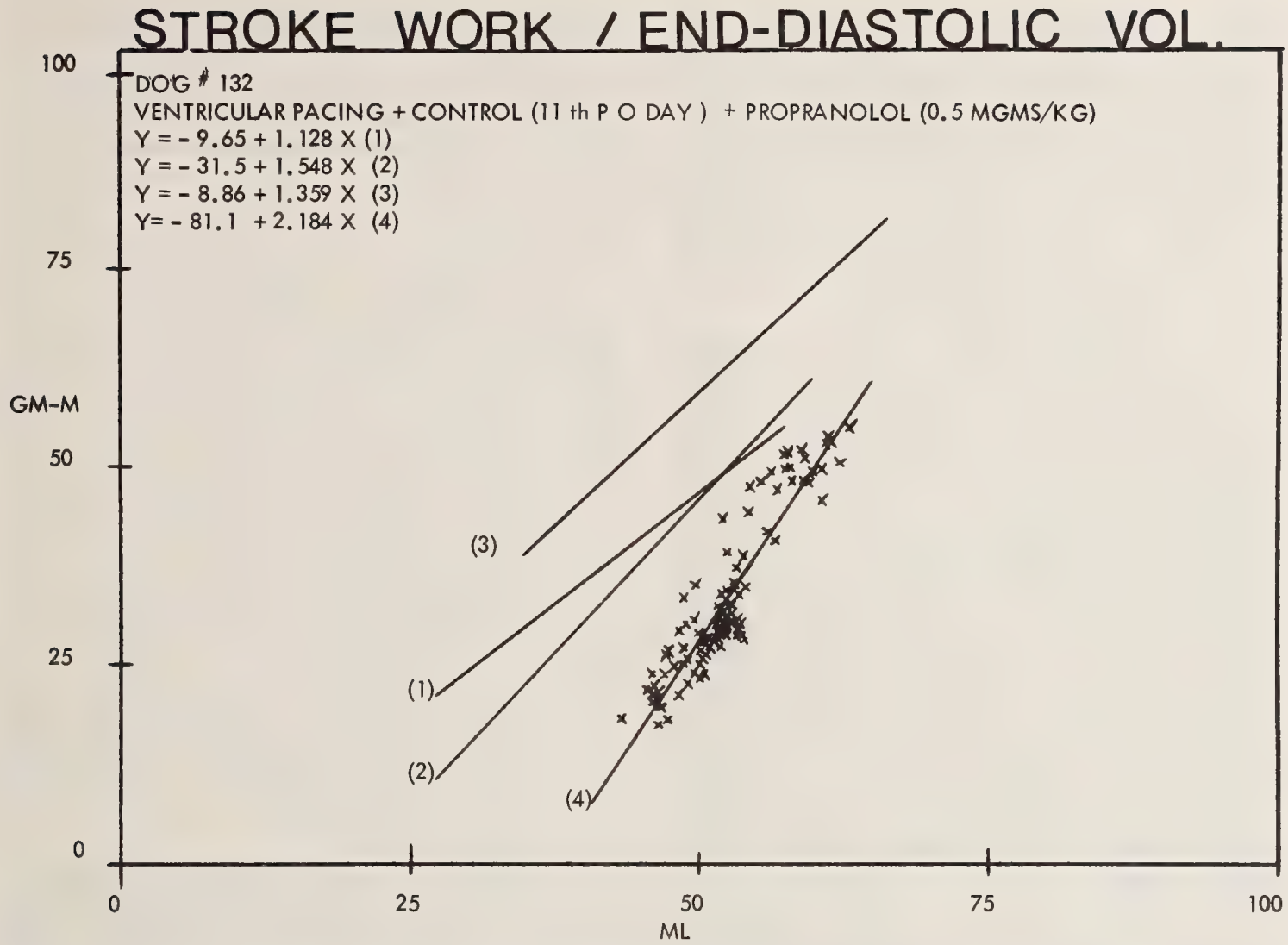


FIGURE 10.—Shows the stroke work/end-diastolic relation after injection of propranolol, and provides the regression lines from the three previous states as shown in Figures 7, 8, and 9.

RAPAPORT, E. The influence of heart rate on left 1963.

30. MITCHELL, J. H., GILMORE, J. P., and SARNOFF, S. J. The transport function of the atrium. Factors influencing the relation between mean left atrial pressure and left ventricular end-diastolic pressure. Amer. J. Cardio. 9:237, 1962.
31. BERGLUND, E., BORST, H. G., DUFF, F., and SCHREINER, G. L. Effect of heart rate on cardiac work, myocardial oxygen consumption and coronary bloodflow in the dog. Acta. Physiol. Scandinav. 42:185, 1958.
32. LEIGHTON, R. F., ZARON, S. J., ROBINSON, J. L., and WEISSLER, A. M. Effects of atrial pacing on left ventricular performance in patients with heart disease. Circulation 40:615, 1969.
33. PARKER, J. O., KHAJA, F., and CASE, R. B. Analysis of left ventricular function by atrial pacing. Circulation 43:241, 1971.
34. DAGGETT, W. M., BIANCO, J. A., POWELL, JR., W. J., and AUSTEN, W. G. Relative contributions of the atrial systole-ventricular systole interval and of patterns of ventricular activation to ventricular function during electrical pacing of the dog heart. Circulation Res. 27:69–79, 1970.
35. SAMET, P., BERNSTEIN, W. H., LEVINE, S., and LOPEZ, A. Hemodynamic effects of tachycardias produced by atrial and ventricular pacing. Amer. J. Med. 39:905–910, 1965.
36. HERD, J. A., and BARGER, A. C. Simplified technique for chronic catheterization of blood vessels. J. Appl. Physiol. 19: 791–792, 1964.

DISCUSSION

G. F. GESTRING, Newark Beth-Israel Medical Center, N.J.: First of all, I would like to congratulate you on your beautiful study and excellent presentation! There is one question I want to put to you. This morning, Dr. Levy showed

us in his "Isorhythmic A-V Dissociation," that the position of the P wave, or atrial contraction, is of major importance. If the atrium contracts prior to the ventricle, the BP increased to nearly double the value than if the contraction occurred after the QRS complex. Yet, in your carefully controlled study a BP comparison of sequential versus ventricular pacing showed no significant difference between both situations. Would you please comment on this?

DR. HAWTHORNE: Well, I don't really know the answer to that. I think that one has to deal with the concept of what he's trying to do when constructing the ventricular function curve. All we really wanted to do is to change the end diastolic size. It doesn't matter what happens, so long as you do not get significant mitral regurgitation so that you really can't estimate the stroke volume. In all of these situations where there was a bizarre relationship between the atrial contraction and the ventricular contraction, the alteration was such that the atrium was contracting against the closed mitral valves so that it did not affect our data. I think that I would, as a physiologist, have to go with sequential pacing because that's nearer the normal in a man.

JOHN KENNEDY, Baylor College of Medicine, Houston, Texas: I rise to compliment the essayist on this really very nicely worked out experimental series and ask if he would hazard a clinical extrapolation of these data? Occasionally, patients with symptomatic bradycardia in whom a trial of pacing is used prior to implantation of a permanent pacemaker exhibit cardiac output falls. I wonder if you can comment on that in light of your own experimental observations?

DR. HAWTHORNE: I would guess that if you are doing ventricular pacing, you do not get the assist from the increase in myocardial contractility, and that might create a problem in terms of cardiac output. I've not thought about that. One says that the cardiac output does not change very much over this wide range, and we've found in these data that it really doesn't. The cardiac output stays pretty level over this range of pacing. I think we were more concerned with whether or not you could use the curve at all. I really don't know how to answer your question.

K. L. GOULD, University of Washington, Seattle: Your last thoughts included measure of wall force. What did you actually measure—tension or wall stress? And, if you measured stress, you would need wall thickness. How did you get that in your very nice preparations?

DR. HAWTHORNE: Fantastic that you'd ask me that question. I measured wall force, if you agree with me that wall force would be Pi times the internal radius, times the pressure, times a conversion factor for pressure, which is 1.36. Wall force I think is defined that way. We were very careful not to indicate that we were measuring stress. We had no measurements of thickness. We believed that one would have to deal with midwall tensile stress which would require that. It would also require you to make other assumptions about geometry. We don't have to make any assumptions if we measure wall force. As far as I know, the way that hoop stress, midwall stresses of various kinds, and wall force all vary together, it is very unlikely that one would see different data if you had measured it any way.

K. SAGAWA, Johns Hopkins, Baltimore, Maryland: Did you do something about reflexing? If not, the direct receptor reflex does not seem to do much, if your ingestation is right.

DR. HAWTHORNE: You're absolutely right. It doesn't seem to do much. I think that if I remember Dean Franklin's paper at the American Heart Meeting, he would agree that it doesn't do much in the conscious dog. His statement is that the carotid mechanoreceptors have their primary effect on peripheral resistance. But I don't think that is all of the story, Dr. Sagawa. I think that here we're dealing with something which is very curious and has a lot more to it. Theoretically, when you get a pacing rate up to 3.5 Hertz, one should at least see the phenomenon. Curiously, pacing the ventricle does not show that. I'm not prepared at this moment to deal with that, but we are trying to work on it.

CHAIRMAN HAMLIN: Did you change the pacing rate suddenly or gradually, and did you see any effect when you changed it gradually?

DR. HAWTHORNE: We monitored these dogs over anywhere from ten seconds to five minute

sample periods; so what we did, Dr. Hamlin, was to change the pacing rate and wait until those variables had stabilized as you could see on those plots. That interval that I showed is approximately a minute and a half. So we did it very slowly. Now immediately upon changing the rate, of course, you do see an increase in peak systolic ventricular pressure and a lot of other things which are maybe the real effect, I don't know. We did not look at it at that time.

ELECTROCARDIOGRAM, MYOCARDIAL ACTION POTENTIAL AND CARDIAC EXCITABILITY IN LABORATORY ANIMALS

E. Lepeschkin*

As the result of personal studies and findings from the literature, a comparison is made between the most important electrocardiographic and cardiac electrophysiologic characteristics in a number of experimental laboratory animals in order to determine which animals are most similar to humans and can therefore be used to study problems arising in human pathology. Reference is made to the trans-membrane action potential, conduction disturbances, the T and U waves, cardiac pacing and defibrillation as well as tissue culture. In the choice of the appropriate experimental animal and method of study, convenience, cost, freedom from artifacts and the possibility of unphysiological experimental conditions must be taken into consideration as well as similarity of function and structure to those of the human heart.

INTRODUCTION**

While studies applicable in all respects to human electrocardiology can be best conducted in humans, it is obvious that certain experimental studies, especially those carrying the risk of cardiac damage, can be carried out only in experimental animals. Certain basic properties of cardiac muscle can be studied with a minimum of cost and equipment in cold-blooded animals, while others require experiments in primates, where the structure of the heart and its position in the thorax closely approximate those of humans. In the following we shall consider which of the commonly used experimental animals are best suited for the study of the most important clinical problems in electrocardiography and cardiac electrophysiology. Because of time limitations we shall confine ourselves to problems involving the ventricles.

* Department of Medicine, University of Vermont, College of Medicine, Burlington, Vermont.

** Supported by the U. S. Public Health Service Research Grant HE-01486 and by the Research Career Award HE-K6-440 from the National Heart Institute to Dr. Lepeschkin.

LABORATORY ANIMALS

The Frog

The frog as an experimental animal offers the advantage of low initial cost and upkeep, and because of its low oxygen consumption and respiration through the skin, access to the heart is possible without the inconvenience of artificial respiration. Since the frog ventricle has a sponge-like structure and no blood vessels, strips of ventricular muscle are much less likely to suffer hypoxia than hearts of animals which ordinarily depend on a blood supply through blood vessels. Furthermore, because the heart is normally at room temperature, its surface can be exposed without the distortion of the T wave and S-T segment which is unavoidable when the heart of warm-blooded animals is exposed to the air.

As can be seen from Table I, the resting and action potentials of the frog ventricle are quite comparable to those of human hearts, and the basic ionic mechanisms of excitation and repolarization are similar.[1,3,9] Although the muscle fibers are smaller than those of mammals, they can be more easily penetrated *in situ* because of the less vigorous heart beat. However, the plateau is longer and the T wave more pointed than in man, and because the ventricular action potential is sensitive to acetyl choline, the T wave is much more variable than in mammals.[4] There are no Purkinje fibers and the muscle fibers have no transverse tubules, and the conduction velocity is much slower than in mammals. Because of this the frog heart can be easily used for the study of ventricular fibrillation (this can be done only in the larger mammals).

Another property of frog myocardium in which it deviates appreciably from man and other mammals is the excitability curve. Since

TABLE I.—*Anatomical, chemical and electric characteristics of ventricular muscle in man and some experimental animals*

	Ref.	Man	Dog	Cat	Cow	Pig	Guinea Pig	Rabbit	Rat	Chicken	Frog
Fiber diameter (μ)	1, 2	16	16	16	10	10	13	11	9	5	5
T tubes	2, 11	++	++	++	++	++	++	++	+	—	—
Purkinje penetration	22	60%	60%	60%	100%	95%	?	?	?	100%	—
K muscle*/serum	6, 12, 23–29	18	18	18	11	14	12	12	20		
Na muscle*/serum	6, 12	0.31	0.27	0.28	0.49	0.29	0.40	0.39	0.25		
Ca muscle* (mg%)	12, 23–26	4.6	5.0	5.0	2.5	6.6	6.8	6.7	13.0		3.0
Resting pot. (mV)	1, 2–5, 28 29, 7, 8–10, 25	86	85	88	85	79	83	81	83	64	84
Action pot. (mV)	"	115	105	116	110	106	105	112	101	89	102
Spike	"	—	+	+	—	—	—	—	++	—	—
Plateau	"	+	+	+	++	++	+	+	+—	+	++
Conduct. Vel. (m/sec)	1	1.3	1.0	.96							0.17
Q-aTc	6	100%	90%	95%	130%	120%	115%	115%	25%	100%	150%
Chronaxie (msec)	13, 16–20	0.3**	0.2***								2.0

* In fresh fat-free and blood-free muscle.

** *In vitro* value = 3 ms.[15] The extreme *in vivo* value of 2 ms [19] is out of range of all other findings.

*** *In vitro* value = 1 ms.[14]

the absolute excitation threshold, expressed in terms of voltage or current strength, varies with the electrode area and the characteristics of the volume conductor surrounding the myocardium a better quantitative method applicable under all conditions should measure current density or voltage gradient per millimeter, at the myocardial level.[20] Unfortunately, this method of measurement has not been applied widely, and the term "chronaxie" has therefore been used to characterize the strength-duration curve under all conditions of stimulation. This value indicates the shortest stimulus duration which will cause a response at a current strength twice that needed with a very long stimulus duration (rheobase). We have found [20] that the chronaxie of the frog myocardium is about 2 msec, that of frog skeletal muscle 1.2 msec and that of frog motor nerve 0.1 msec—all values considerably higher than those found with implanted ventricular pacemakers in humans (Table I). The low values in the frog are probably due to the relatively low temperature, since values in the dog or human taken from the cooled surface of the exposed heart or in isolated myocardial strips were found to be lower than in the case of implanted pacemakers (footnotes to Table I).

The Chicken

The chicken as an experimental animal is at present in use only for the study of embryonic muscle in tissue cultures. As in the case of isolated muscle strips, this method has the advantage of accessibility and of avoiding the unpredictable intervention of the homeostatic mechanisms of the body. However, the tissue culture method allows the isolation of muscle cells which are free of nerves and are under natural conditions, without the effects of mechanical injury unavoidable during preparation of a muscle strip. Since the cells arrange themselves in a single layer, they are not likely to suffer from hypoxia. This arrangement also allows exact control of the current distribution during stimulation and of the chemical and physical properties of the extracellular medium, and makes it possible to change these properties rapidly. Furthermore, mechanical contractile and electrical properties can be measured on the same cell while in strips or *in situ* hearts, electrical properties can be measured only in surface cells while the contraction curve is predominantly the result of activity of the inner cell groups, which may be under different physiological conditions than the surface cells.

Chicken embryos are said to have less myocardial potassium and more myocardial sodium than the adult birds, but their action and resting potentials seem to be of a comparable magnitude.[25] One difference compared to mammals is that the muscle cells of birds are smaller in diameter and have no transverse tubules (Table I). The absence of these tubules in birds and amphibia is presumably related to the small fiber size, which could provide adequate diffusion

of calcium to the contractile elements without the aid of tubules.[2] Another pecularity of the birds is that the Purkinje fibers follow the coronary vessels and therefore penetrate the myocardium from the epicardium toward the endocardium.[22] This explains the negative QRS complex in ventricular surface leads [4] and could provide an ideal opportunity to study the electrical properties of Purkinje fibers *in situ* under physiological conditions. The fact that birds have the tallest U waves among all experimental animals [4] may be related to this distribution, if the concept that the U wave is the T wave of the Purkinje fibers is correct.

The Rat

Because of its low cost and upkeep the rat has been a favorite object of experimentation concerning the electrical and mechanical properties of heart muscle. Its myocardial cells have a diameter only slightly smaller than that of the larger mammals, and are provided with transverse tubules, though of a smaller size.[11] The resting potential is of the same magnitude but the action potential is lower (Table I) and is characterized by prominent rapid initial depolarization (phase 1), a steep, low plateau (phase 2) and a terminal phase (phase 3) which is less rapid than in other species. Because of this configuration the T wave has two parts: a prominent initial summit, which corresponds to phase 1, and a low, occasionally barely perceptible, terminal summit, which corresponds to phase 3 (Fig. 1). The interval from beginning of QRS to the apex of the tallest T wave, corrected for the heart rate (Q-aTc) is therefore very short. This configuration of action potential and T wave is also present in most other rodents, insectivores, marsupials and chiropterae, which are mostly hibernators and small (a notable exception is the kangaroo).[6]

Coraboeuf [10] found that tetrodotoxin, which inhibits the entry of sodium into the cell, eliminates in the rat the entire initial portion of the action potential and reduces its amplitude considerably. In the guinea pig and other mammals which have a high and relatively flat plateau, the toxin makes the ascent of the action potential slower but does not change its amplitude. Coraboeuf believes that the high plateau, which is believed to be caused by a slow calcium current,[10,28] is absent in the rat because of its high intracellular calcium content, and the peak of the action potential is due entirely to sodium entry. This would also be in keeping with the low myocardial sodium and presumably a high transmembrane sodium gradient in the rat.[4] Because of these peculiarities, the dependence of the myocardial contraction strength on the preceding diastolic interval in the rat is different from other mammals.[5,10] Rats and mice therefore cannot be compared to man in their T wave behavior. An illustration in the "Heart Bulletin" (Vol. 3, No. 3) showing F. N. Wilson, the father of modern experimental electrocardiography, experimenting on a rat, is completely imaginary, since he never used rats for his electrocardiographic studies.

The ungulates have about the same myocardial fiber size and structure as well as resting and action potentials as humans, but show a more horizontal plateau and a longer, more peaked T wave with a later apex (longer Q-aTc interval) (Table I and Fig. 1). This was attributed [6] to the high potassium content of the grass which they eat and a higher K concentration in the serum, resulting in a higher transmembrane K gradient, since increasing the K concentration of the perfusing fluid causes the same changes in configuration. Another reason could be that in all these animals the Purkinje fibers, which have a very long plateau, extend throughout the thickness of the ventricle. This may also be the reason that the T wave tends to be directed opposite the QRS complex, that is, there is less of a gradient in ventricular action potential duration.[4] However, the main differences between man and the ungulates, also caused by the wide penetration of the Purkinje fibers, are that in the ungulates QRS is much narrower for the heart size, that ventricular activation is mainly radial and that bundle branch block does not result in typical changes of QRS found in man or the carnivora. The pig seems to show all these characteristics to a smaller degree than the other ungulates, as reported by Dr. Hamlin.

The rat embryo has also been used extensively for tissue cultures, which in this case are more

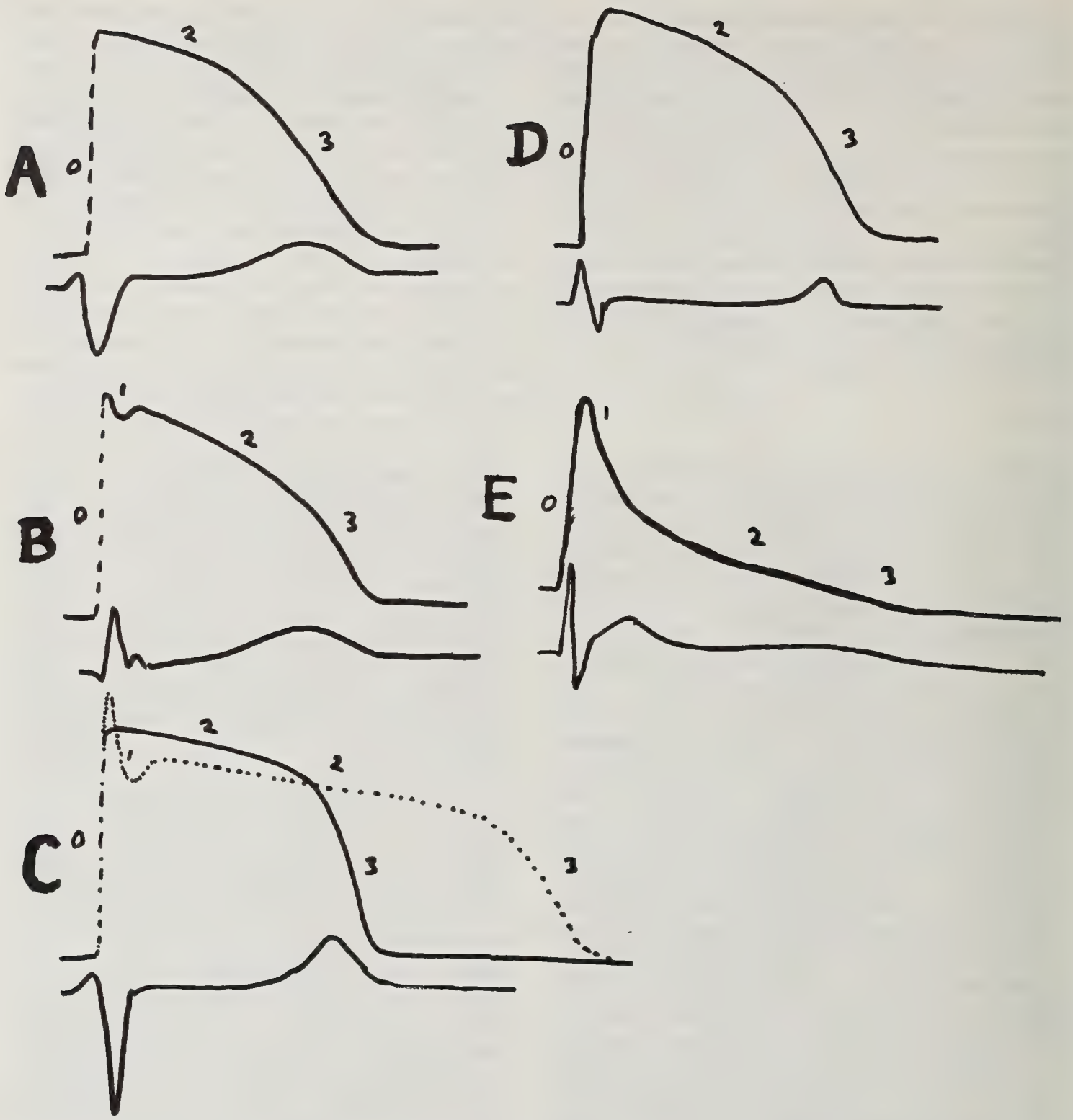


FIGURE 1.—Form of the ventricular action potential and electrocardiogram in different species. Phases of the action potential: 0 = rapid depolarization; 1 = rapid initial repolarization (spike); 2 = plateau; 3 = rapid terminal repolarization.

A: Human right ventricular trabecula carnae (constructed from data of Trautwein et al.[32]). Below, typical human ECG.

B: Perfused cat heart, synchronous with electrogram from aortic cannula to apex (from personal data).

C: Perfused pig moderator band with synchronous Purkinje action potential (constructed from data of Gettes et al.[27]). Below, typical pig ECG.

D: Guinea pig heart in situ, with synchronous ECG (constructed from data of Gargouïl[33]).

E: Rat heart in situ, with synchronous ECG (constructed from data of Gargouïl[33]).

difficult to prepare than those from the chicken embryo but have the advantage of being more similar to human myocardium as far as cell structure is concerned. The steep plateau and early T wave of the rat are not present in the rat embryo and fetus,[9] and the action potential is therefore similar to that of higher mammals and man.

Guinea Pigs, Rabbits

The guinea pig and the rabbit are the only members of the rodent family that show the same high and more horizontal plateau as man and the carnivora. This was attributed to the fact that they are the only grass-eaters among the rodents.[6] The myocardial electrolyte composition is also more similar to the latter (Table I). The T wave of the rabbit and guinea pig is also quite similar in its polarity to that of man, usually opposite to QRS in direction[4] and also shows the same changes as the result of drugs and electrolyte imbalance as the human T wave.[5,6] The isolated rabbit heart, perfused through the coronary arteries and surrounded by a temperature-controlled shield, was chosen for such studies because it is under more physiological conditions than a muscle strip, changes in the perfusing solution reach all parts of the ventricle rapidly and unpredictable reactive changes in blood composition appearing in a heart *in situ* can be avoided. Although only physically dissolved oxygen can reach the heart, the lower viscosity of the solution as compared to blood causes an increase in coronary flow which makes up for the smaller amount of oxygen carried by a unit volume. Better visibility and freedom from potassium liberation through hemolysis are other advantages of a nutrient solution compared to whole blood perfusion. Some workers[10] have kept the temperature of the heart low (at 24–30°C) in order to reduce the oxygen consumption, but such low temperatures may interfere with the sodium pump. It should be mentioned that because of its less forceful contraction the guinea pig heart is easier to penetrate with the flexibly suspended micro-electrode than the rabbit heart.

It has been mentioned that the rapid ascent of the action potential of the guinea pig is followed by a slower ascent to a rounded plateau which was attributed to a slower entry of calcium or to a separate slow sodium channel.[10,28] The action potential of rabbits, ungulates and human myocardial strips[32] shows a slow component at the end of its ascending branch while that of the cat and dog always shows a spike (Table I). In the guinea pig heart *in situ* this slow portion can be the result of a superposition of the potentials caused by activation of distant regions of the heart,[7] and the same can be true for an initial spike,[4] which appears when muscle near the reference electrode is activated late. It is, therefore, not clear whether the spikes of the carnivora are the equivalent of phase 1 and caused by sodium entry as in the case of the rat ventricle and the Purkinje fibers, or whether they are caused by superposition of QRS. Their narrow form would be in favor of the latter interpretation.

Cats, Dogs

While the cat and dog have shown little difference compared to man with respect to the resting and action potential, myocardial fiber structure and distribution of Purkinje fibers (Table I), they do differ from man in the direction of the QRS complex and T wave. Dogs often show negative T waves not only in lead I and/or lead II but also in unipolar leads from the regions of the chest which face the surface of the left ventricle. Recently[30] we have measured the myocardial temperatures in the wall of both ventricles by pulling a fine thermocouple junction through the intact chest, lungs and heart in twelve dogs, and have found a good positive correlation between the endo-epicardial temperature gradient in a given section of the left ventricle and amplitude of the T wave in unipolar leads from the overlying region of the chest. In dogs with inverted T waves this gradient was small. In addition to these physiological differences, the T wave may become negative in leads I and II even if it is positive in precordial leads, because the mediastinum of the dog is very loose, allowing the heart to show considable clockwise or counterclockwise rotation with changes of the body position. Baboons, who are dog-like in their chest structure, may also show these labile T waves, but chimpanzees show T

waves which are comparable to those in humans in all respects. Another major difference between dogs and humans is that in the former the right ventricle is cranial to the left while in man it is at the same level or slightly caudal. Since the first experimental studies on bundle branch block were made in dogs and only the longitudinal lead was registered this has resulted in the famous confusion between right and left bundle branch block patterns which persisted for twenty years until F. N. Wilson ended it by introducing precordial leads. In primates the bundle branch block patterns are quite comparable to those in humans.[21]

The electrocardiogram of the cat shows less variability of the T waves than that of the dog, and unipolar leads from the left regions of the chest facing the apex of the left ventricle have in our experience always shown upright T waves, while those facing the lateral wall of the left ventricle may show negative or diphasic T waves. We have not studied the intramyocardial temperature gradients in cats because of the relatively small size of their hearts compared with the thermo-couple junction, but we have studied in 9 normal cats and 24 cats, subjected to epinephrine infusions, the content of potassium, sodium, calcium and magnesium in the endocardial and epicardial half of the left ventricular wall.[26] There was almost a straight-line correlation between the polarity of the T wave in the two locations mentioned above and the difference in the K/Na ratio between the subendocardial and subepicardial halves of the left ventricular wall facing these locations. The correlation with the other electrolytes was less pronounced. The same correlation was present in 56 patients who died soon after an electrocardiogram was taken and in whom we determined the concentrations of these electrolytes in the subepicardial and subendocardial halves of the left ventricular wall.[31] Since the duration of QRS is much greater in humans than in cats it is necessary to compare the ventricular gradient (QRST area) rather than the absolute amplitude of the T wave with the transmural electrolyte gradients. Also, the location of the precordial leads did not exactly match that of the myocardial regions studied since we compared lead V_5 with the findings at the left ventricular apex, lead V_3 with those at the septum and lead aVL with those of the left ventricular base. Nevertheless, the agreement between the findings in cats and humans justifies comparison between these species with respect to the T wave.

A final problem concerns the comparison of the electrocardiographic limb leads and the so-called unipolar leads in humans and experimental animals. In humans the heart lies in the same plane as the origins of the arms and legs, and this is approximately true of the primates. However, the baboons and the marmosets have a more elongated thorax, and in these monkeys the origin of all four extremities is considerably posterior to the heart. The central terminal in these monkeys reflects the back potentials and the leads taken with it are not truly "unipolar". In an unpublished study with Dr. J. E. Craighead we found that the difference between the findings in the limb leads and the more nearly orthogonal vectorcardiographic systems of Frank and McFee were considerable. In the Iris monkeys, whose thorax is more barrel-shaped, these differences were much less pronounced. Similarly, there should be a considerable difference between the limb and vectorcardiographic leads in dogs with narrow chests (collies, terriers and German shepherds) than in dogs with square chests such as beagles, and in cats which also have a more rounded thorax cross-section. These differences should be even greater in cattle and in birds, where the thorax cross-section is very elongated and the cranial extremities originate near the posterior chest wall.

SUMMARY

In the choice of an experimental animal for the study of cardiac electrophysiology, the frog and turtle have the advantage of low cost and upkeep and of a low oxygen consumption which makes it easier to avoid hypoxia during *in-vitro* studies of basic properties such as transmembrane potential, effects of injury and excitation-contraction coupling, which are fairly similar to those in the human heart. However, findings concerning excitability, activation and repolarization of the heart are not comparable to those

in humans. The mouse and rat also have the advantage of low cost and upkeep and are comparable to humans as far as excitation of the heart is concerned but the repolarization process and the T wave as well as the excitation-contraction coupling seem to depend on a different ionic mechanism than in the human heart and are not comparable to the latter. The rabbit and guinea pig show the greatest similarity to humans in these respects. The cat and dog are also fairly similar to man with regard to these characteristics, but may show considerable differences as far as repolarization sequence and T waves are concerned. The ungulates have the advantage of a larger heart size but their sequence of ventricular activation is completely different, and the results concerning intraventricular conduction disturbances in these animals do not apply to man. In all these animals the position of the heart is different from man, and findings in the limb lead electrocardiogram cannot be compared to those in humans. The only animals which are completely similar to man in all respects are those primates which normally have an upright body position.

REFERENCES

1. Weidmann, S. and Trautwein, W. Electrical properties of cardiac tissue in *Respiration and Circulation*, Fed. Am. Soc. Exp. Biol. Bethesda, Md. 1971, pp. 270–274.
2. Sommer, J. R. and Johnson, E. A. Comparative ultrastructure of cardiac cell membrane specializations. Am. J. Cardiol. 25:184–203, 1970.
3. Ware, F. Effects of calcium deficiency and excess on transmembrane potentials in frog heart. Am. J. Physiol. 201:1113–1119, 1961.
4. Lepeschkin, E. *Modern Electrocardiography. Vol. I: The QRSTU Complex.* Williams and Wilkins, Baltimore, 1951; Reprinted by Edwards Bros., Ann Arbor, Mich., 1970; 2nd edition in preparation.
5. Hoffman, B. F., Cranefield, P. F., Lepeschkin, E., Surawicz, B. and Herrlich, H. C. Comparison of cardiac monophasic action potentials recorded by intracellular and suction electrodes. Am. J. Physiol. 196:1297–1301, 1959.
6. Lepeschkin, E. The configuration of the T wave and the ventricular action potential in different species of mammals. Ann. N. Y. Acad. Sci. 127:170–178, 1965.
7. Dower, G. E. and Geddes, M. A. True transmembrane potential curve from the surface of guinea pig ventricle: its relation to intrinsic deflection. Am. J. Physiol. 198:975–980, 1960.
8. Hoffman, B. F. and Cranefield, P. F. *Electrophysiology of the heart.* McGraw-Hill Book Company, Inc., New York, N. Y., 1960.
9. Bernard, C., Monnereau-Soustre, H. and Gargouil, Y. M. Evolution, chez le foetus de rat, des potentiels intracellulaires du myocarde ventriculaire. Journal de Physiologie 55:113–114, 1963.
10. Coraboeuf, E. and Vassort, G. Effects of some inhibitors of ionic permeabilities on ventricular action potential and contraction of rat and guinea-pig hearts. J. Electrocardiol. 1:19–30, 1968.
11. Denoit, F. and Coraboeuf, E. Etude comparative d'ultrastructure du myocarde chez le rat et le cobaye. C. R. Soc. Biol. 159:2118–2122, 1965.
12. Clarke, N. E., Lackey, R. W. and Tanz, R. D. Chemical composition of the heart in *Respiration and Circulation*, Fed. Am. Soc. Exp. Biol., Bethesda, Md. 1971 pp. 256–270.
13. Suckling, E. E., Brooks, C. McC., Orias, O., Gilbert, J. L. and Siebens, A. A. Determination of excitability of mammalian heart at intervals throughout cardiac cycle. Am. J. Physiol. 162:213–218, 1950.
14. Moore, E. N., Preston, J. B. and Moe, G. K. Durations of transmembrane action potentials and functional refractory periods of canine false tendon and ventricular myocardium: Comparisons in single fibers. Circ. Res. 17:259–273, 1965.
15. Ullrick, W. C., Lentini, E. A. and Sommers, S. C. Excitability and contractility of postmortem human heart muscle. Laboratory Investigation 6:528–535, 1957.
16. Weale, F. E., Deuchar, D. C. and Nightingale, A. Electrical excitability of the human ventricular myocardium. Guy's Hospital Reports 109:157–159, 1960.
17. van Dam, R. T., Durrer, D., Strackee, J., and van der Tweel, L. H. The excitability cycle of the dog's left ventricle determined by anodal, cathodal and bipolar stimulation. Circ. Res. 4:196–204, 1956.
18. Furman, S., Denize, A., Escher, D. J. W. and Schwedel, J. B. Energy consumption for cardiac stimulation as a function of pulse duration. J. Surg. Res. 6:441–445, 1966.
19. Marchand, P., Jaros, G. G. and Obel, I. W. P. Long-pulse stimuli for cardiac pacing. Ann. N. Y. Acad. Sci. 167:707, 1969.
20. Irwin, D. D., Rush, S., Evering, R., Lepeschkin, E., Montgomery, D. B. and Weggel, R. J. Stimulation of cardiac muscle by a time-varying magnetic field. IEEE Trans. on Mag. Vol. Mag–6:321–322, 1970.
21. Bristow, J. D. and Malinow, M. R. Spontaneous bundle branch block in rhesus monkeys. Circ. Res. 16:210–220, 1965.
22. Robb, J. S. *Comparative Basic Cardiology.* Grune & Stratton, New York & London, 1965.

23. Fizelova, A., Fizel, A. and Sapakova, E. Dynamische Verfolgung der Veränderungen des Elektrolyten- und Wasserstoff-wechsels im Verlauf der progredienten experimentellen Aortenkonstriktion. Zeitsch. Kreislauff. 53:718–724, 1964.
24. Holland, W. C. Ion distribution and myocardial metabolism as affected by cardiac glycosides. Circ. Res. 15:II–85/II–95, 1964.
25. Yeh, B. K. and Hoffman, B. F. The ionic basis of electrical activity in embryonic cardiac muscle. J. Gen. Physiol. 52:666–678, 1968.
26. Kimura, H., Bajusz, E., Herrlich, H. C. and Raab, W. Prenecrotic epinephrine and corticoid-induced myocardial electrolyte shifts. Cardiologia 53:175–191, 1968.
27. Gettes, L. S., Surawicz, B. and Kim, K. H. Role of myocardial K and Ca in initiation and inhibition of ventricular fibrillation. Amer. J. Physiol. 211:699–702, 1966.
28. Paes, de Carvalho, A., Hoffman, B. F. and de Paula Carvalho, M. Two components of the cardiac action potential. I. Voltage-time course and the effect of acetylcholine on atrial and nodal cells of the rabbit heart. J. Gen. Physiol. 54:607–635, 1969.
29. Gettes, L. and Surawicz, B. Effects of low and high concentrations of potassium on the simultaneously recorded Purkinje and ventricular action potentials of the perfused pig moderator band. Circ. Res. 23:717–729, 1968.
30. Lepeschkin, E., Takahashi, N. and Herrlich, H. C. Role of myocardial temperature gradients in the genesis of the T wave. J. Electrocardiol. 2:216, 1969.
31. Lepeschkin, E., Kimura, H. and Raab, W. Correlations between the ventricular gradient (QRST area) and the myocardial gradients of K, Na, Ca and Mg. Proc. Internat. Union Physiol. Sci. 9:1015, 1971.
32. Trautwein, W., Kassebaum, D. G., Nelson, R. M. and Hecht, H. H. Electrophysiological study of human heart muscle. Circ. Res. 10:306–312, 1962.
33. Gargouïl, Y. M. Relations entre l'activité électrique cellulaire et globale du coeur et certains aspects de son métabolisme. Thèses, Fac. Sci. Univ. Poitiers, Série A, No. 34, 1958.

AN ANIMAL MODEL OF ISORHYTHMIC ATRIOVENTRICULAR DISSOCIATION

M. N. Levy*

In patients with atrioventricular dissociation, the atria and ventricles are under the control of presumably independent pacemakers. Yet, several lines of evidence indicate that there is a distinct interaction between these pacemakers. The strongest evidence is that the separate pacemakers may discharge at the same frequency; such an arrhythmia is termed isorhythmic dissociation. By means of a series of experiments on anesthetized dogs, it was possible to determine the mechanism responsible for the synchronization of these seemingly independent pacemakers. Complete heart block was produced acutely by injecting 95% ethanol directly into the A-V conduction tissue. An electrode catheter was inserted into the right ventricle, and the ventricle was paced at a constant rate close to the prevailing frequency of discharge of the S-A node. It was found that the S-A node then assumed the mean frequency of the artificial pacing rate of the ventricles, a condition which simulates clinical isorhythmic dissociation. The S-A nodal frequency was observed to oscillate rhythmically about the constant frequency of ventricular pacing. Hence, the P waves of the electrocardiogram were found to pass periodically from slightly in front of to slightly behind the QRS complexes. When the P waves preceded the QRS complexes, atrial contraction was able to contribute to ventricular filling, and the arterial blood pressure began to rise. Presumably, this stimulated the arterial baroreceptors, which reflexly decelerated the S-A node. This caused the atrial contractions to occur after the beginning of ventricular systole, and therefore arterial blood pressure began to drop. This initiated a reflex acceleration of the S-A node, the P waves again moved in front of the QRS complexes, and the cycle was repeated continuously. Interference with the baroreceptor reflexes by preventing the blood pressure fluctuations or by sectioning the efferent nerves to the heart was found to terminate atrioventricular synchronization. Hence, the baroreceptor reflex appears to be responsible for synchronization of atria and ventricles in this arrhythmia.

INTRODUCTION**

In patients with atrioventricular dissociation, the atria and ventricles are controlled by independent pacemakers. However, it has been recognized for many years that some sort of interaction exists between these two presumably independent pacemakers.[1] Most commonly, this interaction is manifested by a phenomenon which has been termed "accrochage" (from *accrocher*, to hook together). It consists of a transient but recurrent tendency for the frequency of discharge of one of the pacemakers to approach the frequency of the other, such that the two pacemakers discharge temporarily at approximately the same frequency.[2] Hence, the atria and ventricles appear to be locked together briefly.

This tendency toward synchronization of two presumably independent pacemakers may be more persistent, with the result that the atrial and ventricular pacemakers may discharge at the same mean frequency, sometimes for protracted periods of time. Such a condition is termed "isorhythmic A-V dissociation." Despite the same mean frequency, there is indisputable evidence that no direct communication need exist between the two pacemakers. The best illustration is afforded by a patient with complete heart block, in whom an artificial pacemaker has been installed. When the ventricles are paced at a constant frequency which is close to the spontaneous discharge rate of the S-A node, the atrial pacemaker will assume the same mean frequency as that of the artificial ventricular pacemaker.[3] Small changes in the frequency of ventricular pacing will induce equal changes in the spontaneous rate of the natural atrial pacemaker. Confidence that this synchronization of the natural with the artificial pacemaker is not ascribable to a spurious spread of the artificial pacemaker current to the atria is furnished by the observation that the P wave of the electrocardiogram oscillates slowly back and forth across the QRS complex. Hence,

* Department of Investigative Medicine, Mt. Sinai Hospital, Cleveland, Ohio.

** This work was supported by Grant HE-10951 from the U.S. Public Health Service.

the atrial frequency is, at times, slightly greater than the ventricular frequency, and, at other times, slightly less than the ventricular frequency, although the mean frequencies are the same. With surreptitious spread of the artificial pacemaker current, the relationship of the P wave to the QRS complex would probably remain constant.

An example of persistent isorhythmic A-V dissociation which occurred spontaneously in a 44-year old man with chronic myocarditis is illustrated by the cyclic changes in P-R interval which are plotted in Figure 1. In his electrocardiogram (see Figure 5, reference 3), the P wave was usually buried in the QRS complex, or only the very beginning of the P wave was evident just before the onset of the QRS. However, the P wave periodically moved out in front of the QRS, remained there briefly, and then returned to be obscured by the QRS, as shown in Figure 1. During those periods in which the P wave moved to the left relative to the QRS (i.e., those periods of increasing P-R interval), the atrial activation frequency exceeded the ventricular activation frequency, whereas the converse was true when the P wave wandered back toward the QRS. However, on the average, the P and QRS frequencies were equal; i.e., isorhythmicity prevailed.

In this patient, there were changes in the arterial blood pressure which accompanied the periodic variations in the P-P interval (Figure 2). These variations of the blood pressure provide a clue to the mechanism producing the synchronization. It is evident from the figure that when the P wave preceded the QRS complex (left half of the tracing), the arterial blood pressure was significantly greater than when the P wave was obscured by the QRS (right half of the record).

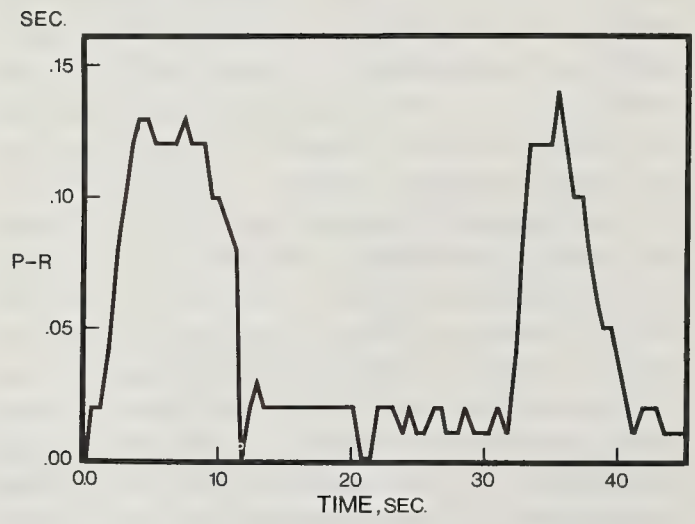


FIGURE 1.—The rhythmic changes in P-R interval which were observed in a 44 year old man with chronic myocarditis, in whom isorhythmic A-V dissociation appeared spontaneously.

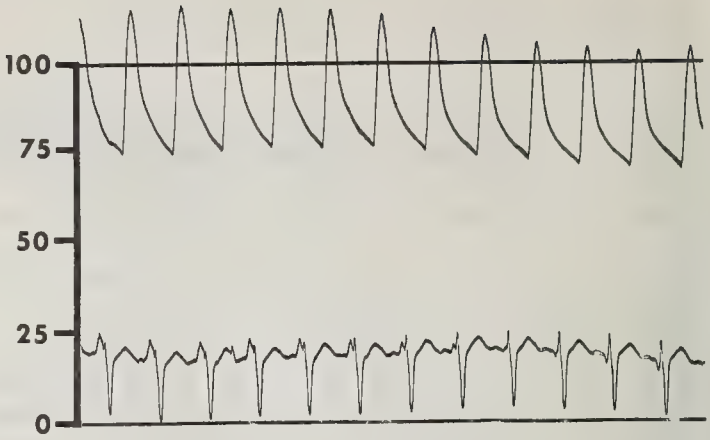


FIGURE 2.—The changes in arterial blood pressure (upper tracing), in mm. Hg, which accompanied the changes in the P-R interval of the electrocardiogram (lower tracing) in the same patient as in Figure 1.

In order to elucidate the mechanism producing isorhythmic A-V dissociation, the clinical condition was simulated by means of an animal model. The model chosen was one in which complete heart block was produced in anesthetized dogs, and the ventricle could be paced at any desired constant frequency.

METHODS

Mongrel dogs were anesthetized with morphine sulfate, 2 mg/kg i.m., followed 30 min later by chloralose, 60 mg/kg i.v. A tracheal cannula was inserted, and artificial respiration was instituted. The chest was opened transversely at the level of the fourth intercostal space. Ethanol, 95%, was injected into the region of the A-V node in order to produce complete heart block.

A bipolar electrode catheter was inserted into the right atrium to permit registration of the atrial electrogram on an eight-channel direct-writing oscillograph (Brush Mark 200). The

arterial blood pressure was also recorded from a brachial artery by means of a Statham P23AA strain gauge.

One series of experiments was conducted on 11 dogs in which the circulatory system was allowed to remain intact, except for third-degree heart block. The ventricles were paced with square wave electrical pulses delivered from a Grass S4 stimulator. The time of each pacing stimulus was recorded on the Brush oscillograph. Also, the atrial electrogram and the pacing stimulus served as inputs to a parallel logic analog computer (EAI 580). The computer calculated the P-P interval (time between atrial activations) and P-St interval (time from the beginning of the P wave to the pacing stimulus) on a beat-by-beat basis, and the results were recorded on the oscillograph. A record taken at fast paper speed is shown in Figure 3. The tall spikes in the upper tracing (labelled P) are the spontaneous atrial activations. Between the P waves are small deflections which represent the current spread of the ventricular pacing stimulus, followed by the QRS complex (recorded from the atrial cavity, and hence much smaller than the P wave). The ventricular pacing stimulus appears in the middle channel, labelled St. The bottom tracing shows the computed P-St intervals, which are recorded at the time of each P wave for the preceding cycle.

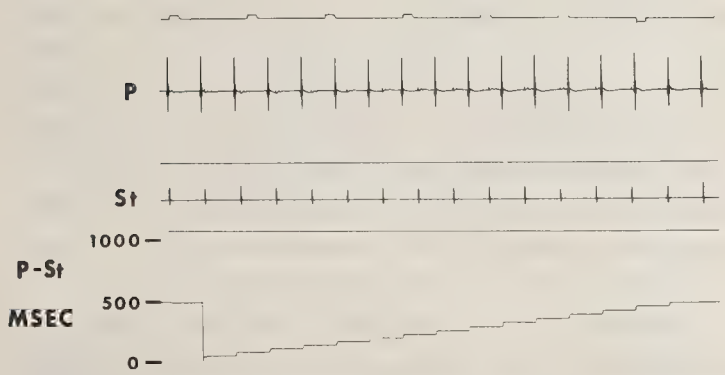


FIGURE 3.—Record from a typical experiment showing the atrial electrogram (labelled P), the ventricular pacing stimulus event marker (St), and the P-St intervals, in msec. The small deflections between the atrial activations (tall spikes) represent the pacing stimulus artifact and the subsequent QRS complex, which are much smaller than the atrial activation deflections since the recording electrodes were located in the right atrial cavity. The P-St interval is recorded at the time of each atrial activation and denotes the P-St interval for the preceding cardiac cycle. The time marker at the top of the record denotes 1 sec intervals.

In each experiment in this first series, the ventricular pacing frequency was varied in a stepwise fashion over a range of values near the spontaneous atrial contraction frequency. In some experiments, observed variations in blood pressure were temporarily attenuated by unclamping a tube which connected a wide-bore cannula in the left subclavian artery to a blood reservoir which was elevated to a hydrostatic level which was approximately equal to the prevailing mean arterial pressure of the animal. In many of these experiments, these procedures were repeated after bilateral vagotomy, bilateral stellatectomy, or both.

In a second series of experiments conducted on 7 animals, the circulatory system was not intact, but the descending thoracic aorta was ligated at the level of the fifth rib. A wide-bore cannula was inserted retrograde just proximal to the ligature, and the blood pressure of the proximal portion of the arterial system was regulated by means of a servo-controlled pump. The regulation of the blood pressure was governed by the analog computer such that the maximum blood pressure was produced at an optimal P-R interval. The shape of the function describing the relationship between the arterial blood pressure and the P-R interval was programmed to resemble that described by Brockman[4,5] for dogs with complete heart block. The analog computer program for accomplishing this has been described previously.[6] The program permits establishment of the optimal P-R interval and the rate of change of blood pressure as a function of the deviation of the P-R interval from this optimal value.

The same computer-generated function could also be used to control the frequency of cardiac neural stimulation. After the procedures involving the changes in blood pressure described above were completed, the vagi were sectioned in the neck, and shielded bipolar platinum electrodes were applied to the distal segments of these nerves. On a beat-by-beat basis, the frequency of vagal stimulation was varied as a function of the deviation of the P-R interval from the optimum value.

RESULTS

In the first series of experiments, when the cardiac nerves were intact, isorhythmic dissociation was evident in every animal when the ventricles were paced at a frequency close to the spontaneous S-A nodal rate. The changes in the P-St (and P-R) intervals during isorhythmic dissociation were very characteristic and resembled those observed in patients with complete heart block and ventricular pacing (Figure 1). In the experimental animals, the P wave wandered rhythmically back and forth across the pacing artifact (St) and subsequent QRS complex. In the tracing recorded at slow paper speed shown in Figure 4, near the left border of the record the P wave was first located just after the St, as denoted by the P-St interval of between 350 and 400 msec. The constant St-St interval was 400 msec; i.e., the ventricle was being paced at a fixed frequency of 2.5 stimuli/sec., or 150 stimuli/min.

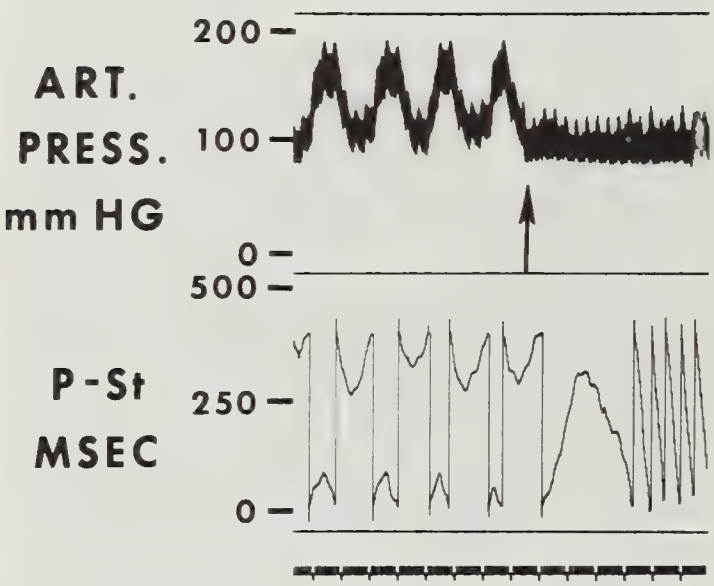


FIGURE 4.—Record from an animal in the first series of experiments (circulation intact, except for complete heart block), showing the changes in arterial blood pressure and P-St interval when the ventricles were paced at a constant frequency of 150 per min. A wide bore cannula in the left subclavian artery was connected by rubber tubing to an elevated blood reservoir. This tubing was clamped to the left of the arrow, and unclamped to the right of the arrow in order to minimize the cyclic blood pressure fluctuations. The sharp vertical deflections downward of the P-St tracing indicate that the P had moved from just in front of, to just behind, the St, whereas the vertical upward deflections denote the crossing of the P and St in the opposite direction. Isorhythmic dissociation is denoted by the rhythmic crossing of the P back and forth across St (to the left of the arrow); loss of synchronization is denoted by the saw-tooth appearance of the P-St tracing (to the right of the arrow). Time scale-vertical deflections denote 10 sec intervals.

With such a large P-St interval (i.e., with the P occurring just after the St, and hence after ventricular activation), the blood pressure was about 100 mm. Hg (left border of Figure 4). When the P moved across the St, as indicated by the first sharp vertical deflection in the bottom tracing, the blood pressure began to rise. As the P-St increased over the next 5 sec to a maximum interval of 80 msec, the blood pressure rose to a maximum value of 170 mm. Hg. Conversely, as the P began to move back again toward St, the diminution in the P-St interval was accompanied by a fall in blood pressure. As the P moved across St, the P-St tracing was deflected sharply upward again. It may be noted in the figure that the blood pressure continued to decline as the P moved behind St (denoted by the P-St intervals > 250 msec).

In Figure 4 to the left of the arrow, the P wave oscillated rhythmically about the St (and QRS), with a period of about 20 sec (i.e., about 3 times/min). An increase in the P-St interval denoted that the S-A nodal discharge frequency temporarily exceeded the fixed ventricular pacing frequency, whereas a decrease in the P-St interval signified the opposite condition. The rhythmic oscillation of the P about the St indicated that the mean frequency of the atrial pacemaker was equal to the fixed frequency of the artificial ventricular pacemaker; i.e., isorhythmic A-V dissociation prevailed.

At the arrow in Figure 4, the clamp was removed from the tubing which connected a cannula in the left subclavian artery with an elevated blood reservoir. This resulted in a marked attenuation of the rhythmic oscillations of the arterial blood pressure. Concomitantly, the rhythmic oscillation of the P wave about the St ceased; i.e., the P-St interval first increased progressively for about 10 sec. until it reached a maximum of about 300 msec., and then it decreased continuously thereafter. Near the right border of the figure, the five vertical upward deflections of the P-St tracing, with slower step-

wise downward deflections indicate that there is a fairly constant difference between the spontaneous frequency of the S-A node and the fixed ventricular pacing frequency; i.e., the A-V dissociation is no longer isorhythmic.

A similar phenomenon in the experiments of the second series (arterial system not intact) is shown in Figure 5. To the left of arrow 1 and to the right of arrow 2, the tubing connecting the cannula in the descending thoracic aorta with the servo-controlled pump was clamped. Hence, no attempt was being made to regulate the arterial blood pressure. The ventricular pacing frequency had been adjusted to a value near the spontaneous S-A nodal frequency in this dog with complete A-V block. It is evident that isorhythmic dissociation prevailed in the left and right thirds of the figure, as evinced by the rhythmic oscillations of the P wave about St. This is manifested in the lower tracing of Figure 5 by the characteristic variations in the P-St interval. The changes in the P-St interval were accompanied by blood pressure oscillations similar to those noted in the previous figure.

Between the arrows (Figure 5), the clamp was removed and the servo-controlled pump was used to hold the arterial blood pressure constant at a value approximately halfway between the peaks and troughs of the oscillating blood pressure in the left and right thirds of the figure. It is evident that when the blood pressure variations were severely attenuated, the P no longer roamed back and forth across St, but it progressively swept through successive cardiac cycles. This is manifested by the saw-tooth

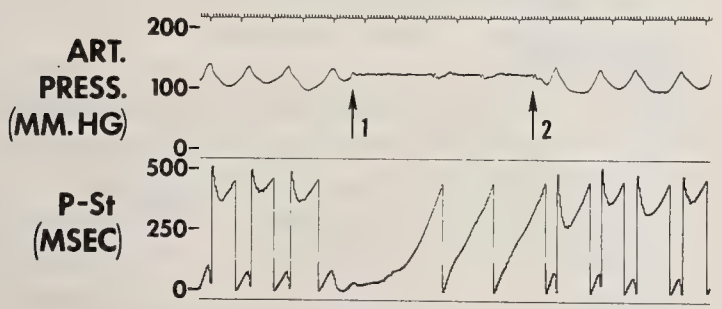


FIGURE 5.—Record from a typical experiment in the second series, in which the arterial system was not intact. Between arrows 1 and 2, the arterial blood pressure was held relatively constant by means of a servo-controlled pump. Remainder of legend as in preceding figure.

appearance of the P-St tracing in the middle third of Figure 5 (between the arrows).

When the cardiac nerves were intact, it was possible to produce isorhythmic A-V dissociation in all animals with complete heart block in this study. When either the vagi or stellate ganglia were sectioned, it was still possible to elicit synchronization, although the range of ventricular pacing frequencies which would still achieve isorhythmicity was diminished. In slightly over half the animals, when both the vagi and stellate ganglia were sectioned, it was no longer possible to elicit isorhythmicity. However, whenever the blood pressure oscillations were severely attenuated, it was never possible to produce synchronization. This was true no matter what the state of the cardiac innervation.

In the second series of experiments in which the blood pressure could be regulated by means of a servo-controlled pump, the importance of the blood pressure oscillations was further elucidated. The blood pressure could be varied as a function of the P-St interval under the control of an analog computer. It was shown in a preceding study[6] that when the cardiac nerves were intact, if blood pressure was altered sufficiently per unit change in the P-St interval, isorhythmicity could be achieved. It was postulated that the blood pressure oscillations evoked A-V synchronization via the baroreceptor reflexes, at least in part.

In order to test this hypothesis, the blood pressure of the dogs in the second series was held constant and the vagi were transected in the neck. Stimulating electrodes were attached to the distal segments of these nerves and the frequency of stimulation was varied as a function of the P-St interval, as described previously.[6] The results of a representative experiment are shown in Figure 6. To the left of arrow 1 and to the right of arrow 2, the vagal stimulation period (reciprocal of the frequency) was made to decrease as the P-St interval increased from 0 to some optimal value, as established by the computer. The changes in the vagal stimulation period which accompany the varying P-St intervals are evident in the lower tracing of the figure. When the P wave moves behind St (as manifested by the high values of

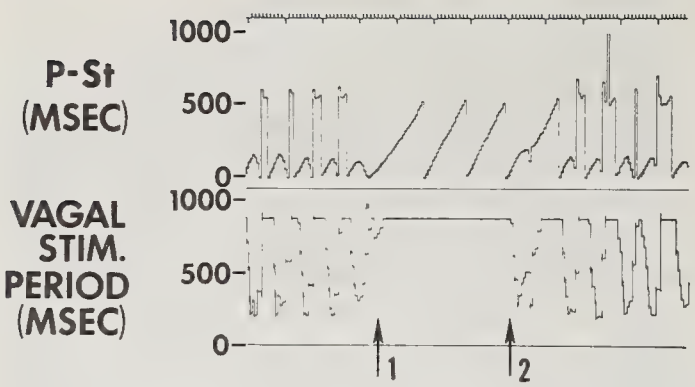


FIGURE 6.—Isorhythmic dissociation produced in a bilaterally vagotomized dog with complete heart block, produced by stimulating the distal vagi at a frequency which varied as a function of the P-St interval. The basal frequency of vagal stimulation was 1.1 pulses/sec (St-St interval, or "vagal stimulation period", of 900 msec), which marks the maximum value of the deflection in the lower tracing. At the optimum P-St interval, the St-St interval was automatically decreased to about 200 msec; i.e., the frequency of vagal stimulation was increased to about 5 pulses/sec. The change in vagal stimulation with changes in P-St interval produced isorhythmic dissociation, as shown by the rhythmic changes in P-St interval to the left of arrow 1 and to the right of arrow 2. When the frequency of vagal stimulation was not altered as a function of the P-St interval (between the two arrows), A-V synchronization ceased, as manifested by the saw-toothed P-St tracing. Time scale-downward deflections indicate 10 sec. intervals.

P-St in the upper tracing), the vagal stimulation period was increased to a constant level of 900 msec. (i.e., a basal stimulation frequency of 1.1 pulses/sec). At the maximum P-St interval observed (about 100 msec) when the P was in front of St, the vagal stimulation period was decreased to about 200 msec (i.e., a frequency of about 5 pulses/sec).

It is evident from the figure that when the vagal stimulation frequency was varied as such a function of the P-St interval, isorhythmicity was achieved (left and right thirds of Figure 6). Between the arrows, the gain of the function was reduced to zero; i.e., vagal stimulation period was held constant at 900 msec. Under those conditions, A-V synchronization ceased, as manifested by the saw-tooth appearance of the P-St tracing. This indicates that there is a sustained difference between the spontaneous S-A nodal frequency and the ventricular pacing frequency.

DISCUSSION

In patients with isorhythmic dissociation, there is frequently a rhythmic variation in the P-R interval (Figure 1) and this is attended by concomitant changes in the arterial blood pressure (Figure 2). Similar changes in the P-R (or P-St) interval and blood pressure are observed in dogs with isorhythmic dissociation (Figures 4 and 5). That the changes in arterial blood pressure are intimately involved in producing the A-V synchronization is suggested by the consistent observation that when the blood pressure variations are attenuated, isorhythmicity ceases (Figures 4 and 5). It is strongly suspected that the blood pressure is involved in A-V synchronization through reflex mechanisms, at least in part, because (a) synchronization can be elicited by varying the frequency of vagal stimulation as a function of the P-R interval (Figure 6), and (b) when the vagi and stellate ganglia are sectioned, synchronization cannot be achieved in more than half the animals, even when the blood pressure oscillations are permitted to occur.

The principal mechanism producing A-V synchronization in complete heart block is represented by the diagram in Figure 7. In the upper left corner of the diagram are shown the two apparently independent pacemakers, the S-A node and the ventricular pacemaker. In the diagram, the ventricular pacemaker is shown not to be influenced by circulatory conditions, which would apply strictly to the example of an artificial fixed-rate pacemaker. In the case of a natural idioventricular pacemaker or A-V node (as

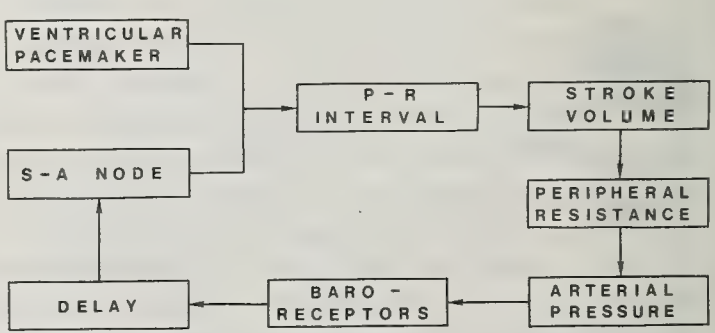


FIGURE 7.—Block diagram denoting the biological feedback control system which causes the variable frequency atrial pacemaker (S-A node) to become synchronized with a fixed (relatively or absolute) ventricular pacemaker in A-V dissociation.

in the patient represented by Figs. 1 and 2), the hypothesis would apply so long as the influence of the critical circulatory conditions on the ventricular pacemaker differed quantitatively from that on the S-A node. The difference in the timing of the initiation of impulses by the two pacemakers is, by definition, the P-R interval. The atrial contribution to ventricular filling depends upon the P-R interval. This dependence has been defined in dogs with complete heart block by Brockman.[4,5] In patients or experimental animals with a constant ventricular rate, the stroke volume and total peripheral resistance determine the level of the arterial blood pressure. Finally, the arterial blood pressure has a reflex influence on the rate of the S-A node, mediated by the arterial baroreceptors. The time from the change in P-R interval till the sympathetic and vagal neurotransmitters affect the S-A node involves an appreciable delay. This time lag plays a role in the rhythmic oscillations of the P wave about the QRS complex, as explained below.

The feedback system illustrated in Figure 7 facilitates the comprehension of the mechanism responsible for A-V synchronization in isorhythmic dissociation. At the left border of Figure 4, for example, the P wave lies behind St. Hence, atrial contraction makes no contribution to ventricular filling. As the P moves in front of St (to the right of the first vertical deflection in the lower tracing), the atrium begins its contraction before the ventricle, and the ventricular stroke volume begins to increase. This is reflected by a rise in the arterial blood pressure. As the pressure increases, the baroreceptor reflex begins to decelerate the S-A node. The P begins to advance more and more slowly ahead of the St, accompanied by a progressive elevation of the blood pressure. Ultimately the S-A nodal frequency becomes equal to the ventricular pacing frequency momentarily. This is the maximum P-St interval recorded while the P wave precedes St (between the first and second vertical deflections in the lower tracing). The S-A node continues to decelerate, so the P wave then begins to move back toward St; i.e., the P-St interval decreases from the maximum value. As the P-St interval diminishes, the blood pressure begins to fall. As the P crosses St (signalled by the second vertical deflection in the lower tracing) and then lies behind St, the atrial contraction occurs after ventricular systole, and the blood pressure continues to fall. This reflexly accelerates the S-A node, with the result that the P moves behind St at a decreasing rate until a minimum P-St interval is reached (between the second and third vertical P-St deflections). At the minimum P-St deflection, the S-A nodal frequency again briefly equals the ventricular pacing frequency. With continued acceleration of the S-A node at the lowered arterial pressure, the P wave again begins to move back toward the St from behind. When it again passes in front of St, the blood pressure begins to rise, and the cycle is repeated over and over again.

In the feedback loop depicted by Figure 7, if there were no delay in the system, the arterial pressure would stabilize at the value which reflexly produced that S-A nodal frequency which equalled the fixed ventricular pacing frequency. This would be achieved at some specific P-St interval. Once this was reached, A-V synchronization would be sustained at a steady arterial pressure and P-St interval.[7] Because of the delay, however, the level of cardiac efferent neural activity is appropriate not to the prevailing P-St interval, but to that existing at some previous point in time (equivalent to the delay in the system). Thus, the system "hunts" for the appropriate P-St interval, and in the process overshoots and undershoots the appropriate value repetitively.

The role of the arterial blood pressure oscillations in achieving A-V synchronization was demonstrated by the failure to produce isorhythmicity when the blood pressure oscillations were attenuated (Figures 4 and 5). This was a consistent observation. However, the presence of an intact baroreceptor reflex arc apparently is not essential in all cases, because isorhythmic dissociation could be produced after bilateral vagotomy and stellatectomy in many experiments. The range of ventricular pacing frequencies within which the S-A node would synchronize with the ventricular pacemaker was much smaller, however, indicating that when the reflex arc was intact, the baroreceptor reflex was the principal mechanism. It has been demonstrated that heart rate varies inversely with

the perfusion pressure in the isolated sinus node artery.[8,9] It is likely that this represents the auxiliary mechanism by which blood pressure fluctuations promote A-V synchronization in the absence of an intact baroreceptor reflex arc.

SUMMARY

An animal model of isorhythmic A-V dissociation was developed in anesthetized dogs. Complete A-V block was produced by injecting 95% ethanol into the A-V node, and the ventricles were paced at a rate close to the prevailing discharge frequency of the S-A node.

Isorhythmicity occurred, such that the mean S-A nodal frequency became equal to the frequency of ventricular pacing. A-V synchronization persisted when the pacing frequency was varied over a small but definite range of frequencies.

A-V synchronization was characterized by a periodic oscillation of the P wave back and forth across the QRS complex and a concomitant rhythmic variation of the arterial blood pressure.

Whenever the amplitude of the blood pressure oscillations was sufficiently attenuated, A-V synchronization ceased. Also, in more than half of the experiments, A-V synchronization could not be achieved after acute cardiac denervation.

Isorhythmicity could be evoked by varying the arterial blood pressure or by stimulating the vagi as appropriate functions of the P-R interval.

The principal mechanism for producing A-V synchronization in isorhythmic dissociation involves the following biological feedback control system: (a) the difference in timing of the atrial and ventricular pacemakers determines the P-R interval, (b) the P-R interval affects the stroke volume, (c) the stroke volume affects the arterial blood pressure, (d) the arterial blood pressure is the stimulus for the baroreceptor reflex, (e) the baroreceptor reflex differentially affects the frequencies of the atrial and ventricular pacemakers, and (f) this changes the phase relationship between the two pacemakers, to close the control loop.

REFERENCES

1. Marriott, H. J. L. Atrioventricular synchronization and accrochage Circulation 14:38, 1956.
2. Segers, M., Lequime, L., and Denolin, H. Synchronization of auricular and ventricular beats during complete heart block. Amer. Heart J. 33:685, 1947.
3. Levy, M. N., and Edelstein, J. The mechanism of synchronization in isorhythmic A-V dissociation. II. Clinical studies. Circulation 42:689–699, 1970.
4. Brockman, S. K. Dynamic function of atrial contraction in regulation of cardiac performance, Amer. J. Physiol. 204:597, 1963.
5. Brockman, S. K. Cardiodynamics of complete heart block. Amer. J. Cardiol. 16:72, 1965.
6. Levy, M. N., and Zieske, H. Mechanism of synchronization in isorhythmic dissociation. I. Experiments on dogs. Circ. Res. 27:429–443, 1970.
7. Levy, M. N., and Zieske, H. Mechanism of synchronization in isorhythmic A-V dissociation. III. Computer model. Circ. Res. 28:23–33, 1971.
8. Hashimoto, K., Tanaka, S., Hirata, M., and Chiba, S. Responses of the sinoatrial node to change in pressure in the sinus node artery. Circ. Res. 21:297, 1967.
9. James, T. N. Pulse and impulse in the sinus node. Henry Ford Hosp. Med. J. 15:275, 1967.

DISCUSSION

L. R. Castillo, Miami Heart Institute, Miami Beach, Florida: Which type of pacemaker did you use for this experiment, a fixed rate or a demand pacemaker?

Dr. Levy: A fixed-rate pacemaker was used in the animal experiments. Actually, for the last several experiments that you saw on the slides, we were using the analogue computer itself for the pacemaker. In the earlier experiments we used just a Grass stimulator, and, of course, it would be fixed rate, but we would change the frequency. We would change it and let it stay at a new frequency. But this was not a demand pacemaker. The patients that we studied were all awaiting the insertion of a permanent pacemaker but we studied them at the time that they had the temporary fixed-rate pacemaker in place.

Colin Grant, Albany Medical College, N.Y.: I may have missed some of the things that you said, but what effects did you find from pharmacological interventions in clinical material?

Dr. Levy: We didn't do any pharmacological interventions in the patients. We did give some atropine in one patient and the results were

equivocal. We did, of course, use some pharmacological interventions in the animals, but even there the results were not too good. In most of the experiments, if we cut the vagi and removed the stellates, we would block synchronization. In a little more than half of the experiments, we were not able to produce synchronization. But, in some experiments we were able to get synchronization even when the vagi and stellates were cut. I might emphasize that whenever we held blood pressure constant, we were never able to produce synchronization. In a little less than half of the cases, if we did an acute denervation of the heart, we could get some synchronization. It was very, very touchy. We had to adjust the pacemaker in the ventricle, or the frequency of the pacemaker, very, very carefully to get it.

You may, or may not, be familiar with the observation that was reported by both Hashimoto and Chiba and by James, that if you change the pressure in the S-A node artery, the heart rate changes inversely as a function of the pressure in the S-A node artery. So, apparently there is a sort of localized baroreceptor reflex within the S-A node itself. And this probably accounts for the synchronization even in the absence of the intact reflex pathway. We tried to use propanolol to block the sympathetics in some of our experiments, but that was unsatisfactory because the heart rate wouldn't stay constant enough. That is, we would get a gradual change in heart rate. The animal would then go into synchronization, but pretty soon because of this gradual shift in heart rate, it would go out of synchronization. So we found that surgical interruption of the reflex paths for our purposes was more satisfactory than using pharmcological agents.

WALTER EHRLICH, Johns Hopkins, Baltimore, Md.: Would you explain how you produced the heart block? Did you cut, or was the procedure acute? Was it performed on an opened chest dog?

DR. LEVY: We pushed the wall of the right atrium over until it was right up against the inter-atrial septum, and then we advanced a small syringe needle into the interatrial septum and injected about 0.2 cc. of 95% alcohol until we produced a complete acute heart block. Yes, it was an open chest dog.

PULMONARY FUNCTION TESTING OF UNANESTHETIZED BEAGLE DOGS

J. L. Mauderly and J. A. Pickrell*

ABSTRACT

Methods for testing pulmonary function were adapted for use on unanesthetized, unsedated Beagle dogs used for studying the effects of inhaled radionuclides. A test apparatus, face mask, and restraining device designed in this laboratory were used for performing comprehensive studies of pulmonary function of untrained dogs.

Ventilation was characterized by measuring respiratory frequency, tidal volume, and minute volume. The open circuit nitrogen washout method used for measuring functional residual capacity also gave indices of intrapulmonary gas distribution such as nitrogen clearance curves and the lung clearance index. Elastic and flow resistive properties of the lung tissue and airways were evaluated by tests of dynamic pulmonary compliance and resistance.

Steady state carbon monoxide diffusing capacity was calculated using alveolar gas tensions obtained by an automatic, volume controlled end tidal sampler. Gas exchange was further characterized by measuring the alveolar-arterial gradients for oxygen and carbon dioxide. Oxygen saturation and acid-base parameters were calculated from femoral arterial samples. Parameters calculated from measurements of expired gas collected during arterial bleeding included oxygen uptake, carbon dioxide elimination, alveolar ventilation, effective ventilation, and alveolar deadspace ventilation.

Tests of lung function during treadmill exercise were used to detect subclinical lung damage. Respiratory frequency, tidal volume, minute volume, gas exchange, and specific ventilation were measured while dogs ran at 3.0 and 4.5 mph.

Normal values are reported for adult Beagles.

INTRODUCTION*

A laboratory designed to evaluate the pulmonary function of Beagle dogs has been developed as part of an AEC-sponsored inhalation toxicology program. Measurements are made to study the quantitative and qualitative relationships between inhalation exposure to various radioactive aerosols and the resulting biological effects. Previous reports[1-5] have indicated the usefulness of the dog as an experimental model for investigations of the effects of external thoracic irradiation on pulmonary function. Measurements, however, were performed with the dog under anesthesia. In order to minimize the risk involved in testing dogs committed to lifespan studies, and to utilize methods of testing similar to those used in human pulmonary function laboratories, procedures were developed which could be applied without the use of anesthetics. This paper describes the pulmonary function testing methods currently used in our laboratory and gives normal values that have been obtained for parameters routinely measured. The procedures are performed without anesthesia or sedation, and, with the exception of those involving treadmill exercise, are performed on untrained dogs.

METHODS

All of the parameters reported, except those obtained during exercise, are measured using an apparatus (Figure 1) fabricated in the laboratory from readily available components. The dogs respire through a low-resistance, low-deadspace breathing valve (No. 1400, Hans Rudolph Inc., Kansas City, Mo.). A system of mechanical and solenoid valves allows inspiration of a choice of gas mixtures and expiration into a laboratory exhaust line, a 30-liter Douglas bag (Warren E. Collins, Inc., Braintree, Mass.), or a 120-liter spirometer (Collins). The dry volume of expirate collected in the Douglas bag is measured by passing the gas through a drying cannister and dry gas meter (American Meter Co., Boston, Mass.). A system of valves allows sampling and analysis of respired gases

* Fission Product Inhalation Laboratories Lovelace Foundation for Medical Education and Research Albuquerque, New Mexico 87108.

** Research performed under U.S. Atomic Energy Commission Contract AT(29-2)-1013.

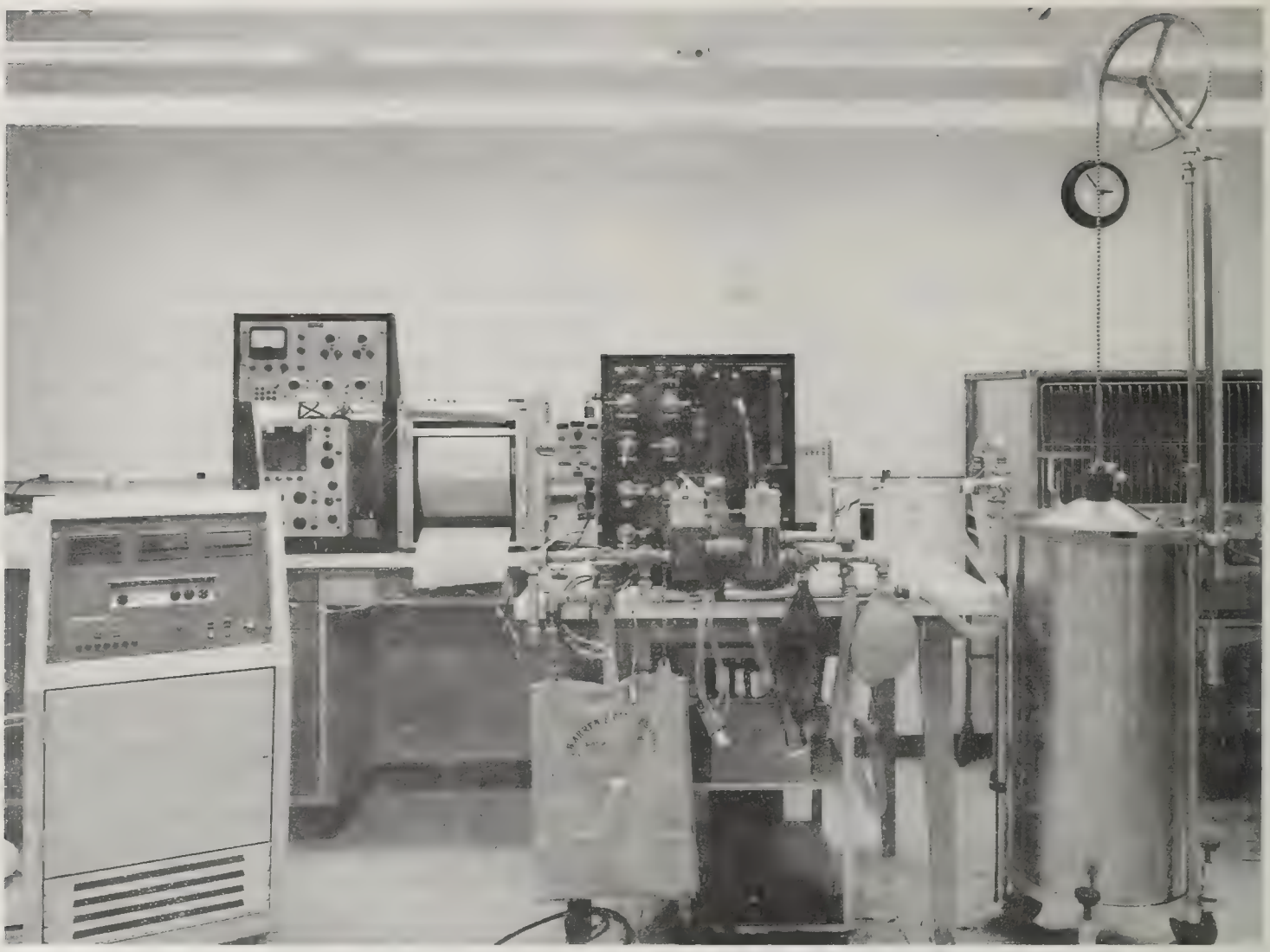


FIGURE 1.—View of apparatus used for measuring parameters obtained at rest.

in the inspired, mixed expired, continuous (breath-by-breath), or end tidal modes. Oxygen, carbon dioxide, and nitrogen concentrations are measured by a mass spectrometer (MS-8, Scientific Research Instruments Corp., Baltimore, Md.), and carbon monoxide is measured by an infrared analyzer (IR-215, Beckman Instruments, Inc., Fullerton, Calif.). Gas concentrations are recorded on a strip chart oscillograph (Visicorder 1912, Honeywell, Denver, Colo.).

For procedures involving intrapleural pressures, the dogs are fitted with the face mask, mouthpiece, and intraesophageal catheter previously described,[6] and for other measurements at rest, only the face mask is used. For testing in the standing position, restraining stocks[7] are used which hold the dog by the legs and muzzle, thereby imposing no restriction upon respiratory movements of the thorax and abdomen (Figure 2).

The dogs are brought to the laboratory in pairs and held in cages to await testing. During one visit to the laboratory, measurements associated with lung mechanics, functional residual capacity, and CO diffusing capacity are performed. On another occasion, parameters associated with arterial blood sampling are obtained, and tests involving treadmill exercise are performed at yet another time. Organization of the procedures in the above manner allows a relatively complete examination of pulmonary function, while requiring the dogs to tolerate the manipulations for only 10 to 15 minutes per session.

Dynamic pulmonary compliance and resist-



FIGURE 2.—Dog in restraining stocks, wearing face mask, and in position for testing.

ance are measured by relating transpulmonary pressure fluctuations to simultaneous respiratory volume and flow rate changes, respectively. Airflow rate is monitored by a pneumotachograph (Vol-O-Flow 10-R-100, National Instrument Laboratories, Rockville, Md.) and differential pressure transducer (PM97, Statham Instruments, Inc., Oxnard, Calif.). Transpulmonary pressure changes are estimated by variations of pressure in the intraesophageal catheter as monitored by another differential pressure transducer (PM5TC, Statham). The reference port of the esophageal pressure transducer is vented to the face mask to eliminate any artifacts caused by the slight pressure drop across the pneumotachograph. These signals are translated into compliance and resistance by an analog computer (794444 R/C Computer, Honeywell). The computer utilizes two subtract circuits which allow the subtraction of either the compliance or resistance component from hysteresis loops produced from the input signals. Subtracted voltages required to flatten the loops displayed on an oscilloscope are used to calculate compliance and resistance by a method similar to that reported by Mead and Whittenberger.[8]

The general technique for performing the open circuit nitrogen washout measurement of the functional residual capacity of Beagle dogs has been reported.[9] The dog is switched at end-expiration from breathing room air to breathing 100% O_2, and the expirate is collected in the spirometer which had previously been flushed with O_2. The washout of nitrogen from the lungs is continuously recorded and when the

end tidal N_2 reaches a stable, minimal level the spirometer valve is closed and the collected gas allowed to equilibrate for 15 minutes. The volume of N_2 contained in the dog's lung at end-expiration and therefore the lung volume at that time, is calculated from the volume and N_2 content of the expirate. The lung clearance index, a means of evaluating the intrapulmonary mixing of inspired gases,[10] is calculated by dividing the liters of gas collected during the washout by the functional residual capacity in liters. When another index of the distribution of ventilation is desired, sequential end tidal N_2 concentrations recorded during the washout may be plotted on semilogarithmic graph paper and analyzed in the manner described by Fowler et al.[11] Respiratory frequency, tidal volume, and minute volume during 100% O_2 breathing are calculated from the N_2 traces and volume collected during the test.

The steady state carbon monoxide method used for measuring diffusing capacity has been recently reported.[12] The dog breathes a mixture of 500 ppm CO in air and a timed collection is made of the expirate. During the collection, end tidal gas samples are collected by a volume-controlled sampling device actuated by changes in intraesophageal pressure. End tidal and expired gas tensions and expired volumes are used to calculate the fraction of CO absorbed by the dog (fractional uptake of CO) and the rate of alveolarcapillary diffusion of CO (diffusing capacity).

Several parameters useful in characterizing gas exchange and ventilatory efficiency are obtained during an arterial blood sampling procedure. The dog is fitted with the face mask, restrained in the supine position, and connected to the test apparatus. With the dog breathing dried room air, breath-by-breath O_2 and CO_2 tensions are recorded and the Douglas bag is flushed with expirate. The skin over a femoral artery is clipped and sprayed with a topical anesthetic-antiseptic solution (Cetacaine^R, Cetylcide Industries, Inc., Long Island City, N. Y.), and a rectal thermometer is inserted. When the recorded gas tension traces stabilize, a timed collection of expirate is begun, during which a blood sample is collected by percutaneous puncture of the femoral artery (Figure 3). Following the gas collection, rectal temperature is recorded and the collected expirate is allowed to equilibrate for 5 minutes, after which the O_2 and CO_2 concentrations and volume are measured. Blood gas tensions and pH of the arterial samples are measured in a manner similar to that previously reported[13] using a PHM 71 analyzer, with a PHA 930 O_2 module, E5046 electrode, and D616 cuvette for P_{O2}, a PHA 931 CO_2 module, E5036 electrode, and D616 cuvette for P_{CO2}, and a G297/G2 glass capillary electrode for pH (Radiometer Corp., Copenhagen, Denmark, and The London Company, Cleveland, Ohio). A computer program developed in this laboratory[14] is used to correct the blood gas and pH values for differences between analytical and rectal temperatures, and to calculate the saturation of hemoglobin with oxygen and acid-base parameters. Alveolar gas tensions are derived by multiplying the recorded end tidal fractions of O_2 and CO_2 by the barometric pressure minus the vapor pressure of water at the individual dog's body temperature, and the alveolar-arterial gas tension gradients are calculated. The gas tensions and volume of the expirate are used to calculate O_2 consumption, CO_2 elimination, and the resulting respiratory exchange ratio. The volume of expired gas and O_2 uptake allow calculation of specific ventilation (the milliliters of ventilation required per milliliter of O_2 uptake). CO_2 output, alveolar P_{CO2}, and arterial P_{CO2} are used to calculate alveolar ventilation, effective ventilation, and alveolar dead space ventilation in the manner described by Luft and Finkelstein.[15] Respiratory frequency, tidal volume, and minute volume during room air breathing are also calculated from data obtained during the bleeding procedure.

Tests utilizing treadmill exercise have been performed on a limited number of dogs trained to run while wearing a latex mask (Figure 4). The mask, constructed by painting an appropriately-shaped mold with liquid latex, has large ports on both sides of the muzzle which are connected to large-bore flexible tubing. One-way valves positioned in the tubing just behind the dog's head allow the dog to inspire room air through a drying cannister, and expire into a 100-liter Douglas bag. Small-bore tubing open-

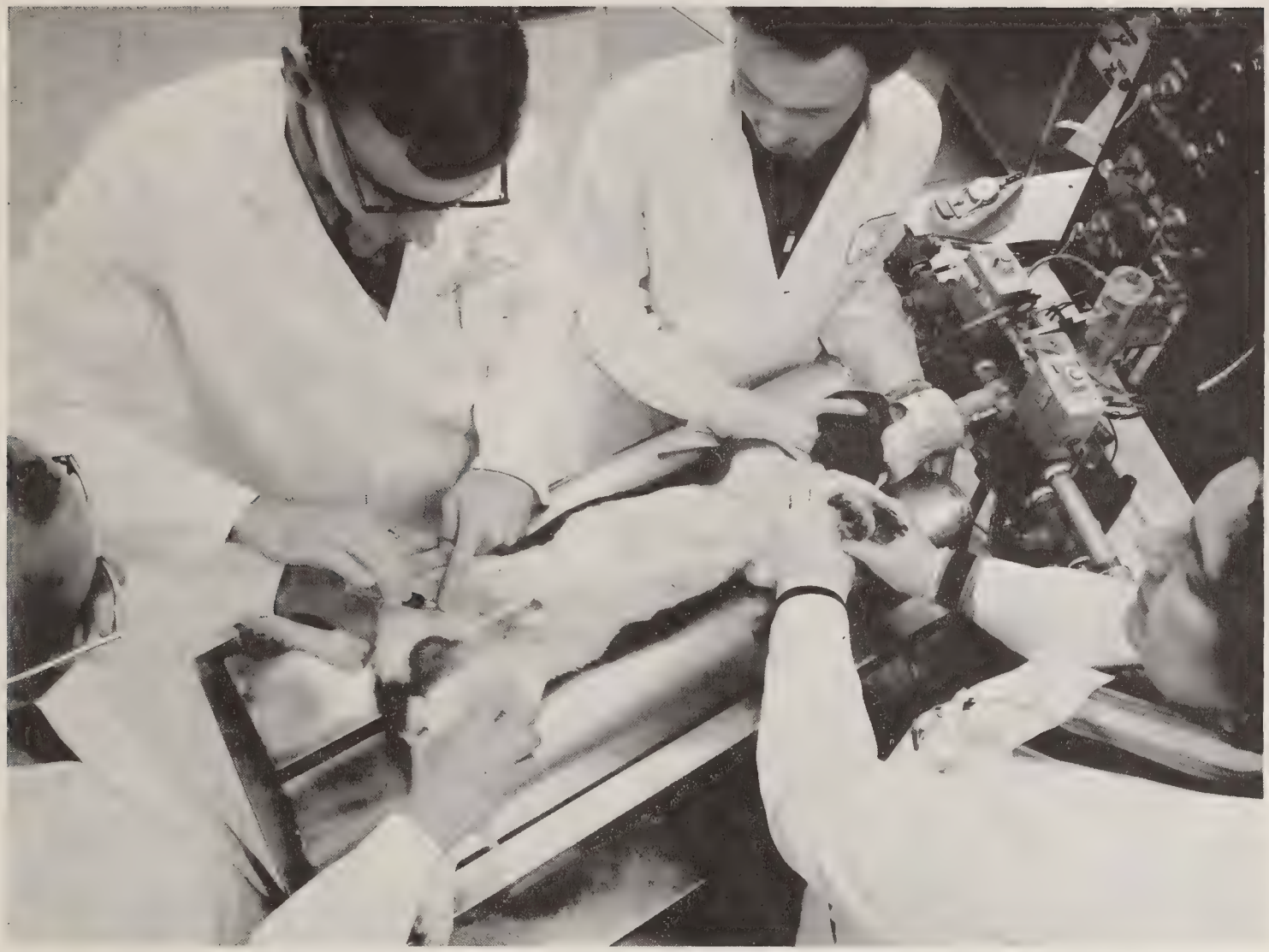


FIGURE 3.—Collection of femoral arterial sample.

ing into the muzzle area of the mask is connected to the inlet catheter of the mass spectrometer, allowing respiratory frequency to be measured from recorded CO_2 traces. The dogs are placed on the treadmill (18-49-D, Quinton Instruments, Seattle, Wash.), fitted with the mask, and allowed to rest quietly for 3 minutes to stabilize the breathing pattern. A timed collection of expired gas is then made, during which the breath-by-breath CO_2 is recorded. Following the collection, the treadmill is started and the dog runs at 3.0 mph for 3 minutes, after which another timed collection is made. The treadmill speed is then adjusted to 4.5 mph, the dog is allowed to run at that speed for 3 minutes, and the expirate is collected a third time. The breathing traces and collected gas tensions and volumes allow calculation of respiratory frequency, tidal volume, minute volume, O_2 consumption, CO_2 elimination, respiratory exchange ratio, and specific ventilation at each speed. The treadmill is kept level throughout the procedure. Speeds higher than 4.5 mph were found to produce a panting breathing pattern, and 3.0 mph was selected as an intermediate speed. The exercise level, while considered only moderate, was found to be one that could be achieved by dogs with significant pulmonary abnormalities.

Measured data for the calculation of most parameters are entered on standardized forms for ease of key punching for computer input and processing. Both the input data and calculated results are placed in a temporary disc file and a printout is returned to the laboratory for validation. After validation, the information is en-

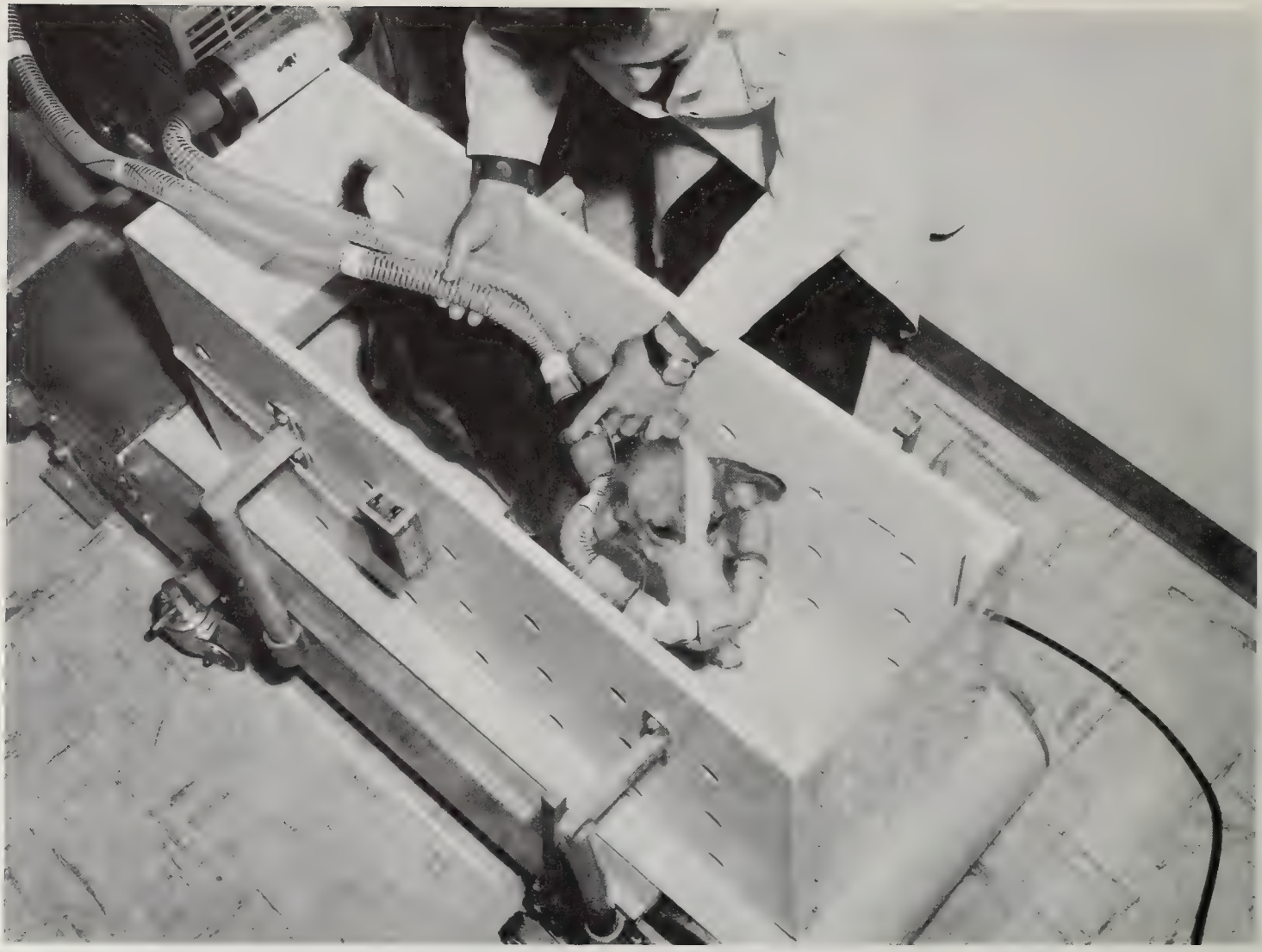


FIGURE 4.—Dog running on treadmill while expired gas is collected.

tered in a permanent record tape file and stored for future recall and analysis.

Measurements were performed on adult, pure-bred Beagle dogs maintained in a closed colony at an elevation of approximately 1680 meters where the barometric pressure was 624 ± 2 mm Hg, resulting in an inspired P_{O_2} of approximately 120 mm Hg.

The means, standard deviations, and ranges of pulmonary function parameters measured at rest were calculated from single measurements on young adult (12 to 14 months) Beagle dogs. If results of the first measurement fell outside the previously calculated normal range,* the dog was retested and the second value, if within the normal range, was included in the data contained herein. Data from equal numbers of male and female dogs were included. Since some of the parameters may not have a normal distribution, and in order to make as few assumptions as possible concerning the distributions, the 2.5–97.5 percentile ranges were calculated by the technique of Herrera.[16]

Single measurements (no re-testing) of pulmonary function at rest were also performed on 10 male and 10 female Beagle dogs, 7 to 10 years old. No attempt was made to select individuals on the basis of physical fitness although none exhibited outward signs of pulmonary distress when presented to the laboratory. Means, standard deviations, and ranges were calculated and the Student T test was applied to determine whether significant differences existed between values obtained from dogs in the younger and older age groups.

* Mauderly, J. L. and Pickrell, J. A., unpublished data.

The pulmonary function of 7 male and 6 female Beagle dogs, 12 to 14 months old, was measured during treadmill exercise. Two or three measurements were performed on each dog, a mean was calculated for each dog, and these means were used to calculate the data given herein.

RESULTS

Table I lists the means, standard deviations, ranges, and 2.5 to 97.5 percentile ranges for pulmonary function parameters measured at rest on 12- to 14-month-old Beagle dogs. Table II lists the means, standard deviations, and ranges for the same parameters measured on 7- to 10-year-old Beagles. Differences between the two age groups significant at the 95% level were found in body weight, dynamic pulmonary compliance and resistance, arterial P_{O2}, the alveolar-arterial O_2 gradient, O_2 saturation of hemoglobin, percent alveolar ventilation, and specific ventilation. Table III lists means, standard deviations, and ranges for pulmonary function parameters of 12- to 14-month-old Beagles measured on the treadmill at 0, 3.0, and 4.5 mph.

DISCUSSION/SUMMARY

Experience in this laboratory had indicated the practicability of evaluating pulmonary function in the unanesthetized dog. The examination of pulmonary function as described herein has been performed on a routine basis for over one year, with individual tests having been in use for various times up to six years. More than 550 dogs have been tested at least once during the past three years and multiple tests were performed on many of them. An estimated one percent of the dogs failed to complete their initial series of tests because of behavioral problems, but only one dog has been rejected for further study because of repeated failure to tolerate the manipulations. It must be recognized, however, that although the dogs in this colony receive no training for pulmonary function testing, they are handled periodically by caretaker, clinical, and investigative personnel from birth until presented to the laboratory for evaluation.

Although data from equal numbers of males and females were used in Tables I and II, no significant sex-related differences in pulmonary function were found in Beagle dogs. Calculations performed on unpublished data have shown that differences apparently related to sex were actually related to body size.

The data listed in Table I are similar to those previously reported[13] except for dynamic pulmonary compliance, for which a mean of 73 ml/cm H_2O was found as compared to the previous mean of 48 ml/cm H_2O. The reason for the difference has not been determined, but may relate to changes in the method of measurement. Compliance in the earlier report was determined graphically by the method of von Neergaard and Wirz,[17] instead of the loop closing method currently in use, and the pressure transducer connected to the esophageal catheter did not have a reference vent to the face mask.

The values listed in Table I for alveolar and arterial P_{O2} and P_{CO2}, the alveolar-arterial graients for O_2 and CO_2, arterial pH, and specific ventilation are similar to values obtained from human subjects at approximately the same altitude. The results of measurements on Beagle dogs (Table I) and values obtained by similar methods from 10 normal, male human subjects with a mean age of 26.5 years (range = 17 to 38 years) were compared (Table IV). The human measurements were performed in duplicate with the subjects breathing room air at rest at an altitude of approximately 1,620 meters (inspired P_{O2} = approximately 122 mm Hg). While slight differences can be seen between the human and dog values, especially in arterial pH where a human mean of 7.45 was found as compared to the dog mean of 7.38, the similarities are striking.

The limited data obtained from older dogs (Table II) would seen to indicate certain age-related changes in pulmonary function similar to those reported for man. A reduction in the oxygen saturation of hemoglobin,[18] and an increase in the alveolar-arterial oxygen gradient[19,20] have also been observed in human subjects. A trend toward loss of diffusing capacity was observed in the older dogs, al-

TABLE I.—*Values Obtained by Single Measurements on Equal Numbers of Male and Female Beagle Dogs Aged 12 to 14 Months*

Parameter	Symbol	Units	No of Dogs	Mean	Standard Deviation	Range	2.5—97.5 Percentile Range
Body weight	wt	kg	100	9.1	1.4	6.6 — 13.6	6.9 — 12.6
Respiratory frequency (room air)	f	breaths/min	60	20	4	10 — 31	11 — 30
Tidal volume (room air)	V_t	ml BTPS*	60	186	52	99 —350	106 —350
Minute volume (room air)	V_E	liters BTPS/min	60	3.72	0.78	2.29 — 6.02	2.31 — 6.00
Respiratory frequency (100% O_2)	f	breaths/min	60	18	6	11 — 28	11 — 25
Tidal volume (100% O_2)	V_t	ml BTPS	60	184	35	119 —247	122 —247
Minute volume (100% O_2)	V_E	liters BTPS/min	60	3.31	0.75	2.12 — 5.71	2.15 — 5.42
Dynamic pulmonary compliance	$C_{L\ dyn}$	ml/cmH_2O	100	73	24	36 —125	38 —120
Dynamic pulmonary resistance	$R_{L\ dyn}$	cmH_2O/liters per sec	100	10	8	1 — 40	2 — 37
Functional residual capacity	FRC	ml BTPS	100	434	83	277 —670	287 —612
Lung clearance index	LCI	liters V_E/liter FRC	60	22.5	4.8	14.8 — 35.7	15.2 — 35.5
CO diffusing capacity	$D_{L_{CO}}$	ml/min/mm Hg	100	9.0	3.1	2.9 — 16.6	3.7 — 15.3
Fractional uptake of CO	Fu_{CO}	decimal fraction	100	0.480	0.105	0.194— 0.749	0.259— 0.660
Alveolar O_2 tension	PA_{O_2}	mm Hg	100	83	5	71 — 97	73 — 92
Arterial O_2 tension	Pa_{O_2}	mm Hg	100	75	5	64 — 90	65 — 87
Alveolar-arterial O_2 gradient	PA_{CO_2}	mm Hg	100	8	3	1 — 15	2 — 14
Arterial CO_2 tension	Pa_{CO_2}	mm Hg	100	41	3	31 — 50	34 — 47
Alveolar CO_2 tension	$PA\text{-}a_{O_2}$	mm Hg	100	37	3	27 — 45	30 — 40
Arterial-alveolar CO_2 gradient	$Pa\text{-}A_{CO_2}$	mm Hg	100	4	1	0 — 7	1 — 7
Arterial pH	pH	mm Hg	100	7.38	0.03	7.31 — 7.45	7.33 — 7.45
O_2 saturation of arterial hemoglobin	SO_2	%	100	91	2	86 — 94	88 — 94
O_2 consumption	V_{O_2}	ml STPD†/min	60	90	22	54 —169	55 —155
CO_2 elimination	V_{CO_2}	ml STPD/min	60	85	22	50 —155	51 —154
Respiratory exchange ratio	RER	V_{CO_2}/V_{O_2}	60	0.95	0.09	0.78 — 1.16	0.79 —1.13
Specific ventilation	V_E/V_{O_2}	ml V_E-ml V_{O_2}	60	40	6	28 — 57	29 — 54
Alveolar ventilation	% V_A	% of total V_E	60	57	6	45 — 71	46 — 70
Effective ventilation	% V_{eff}	% of total V_E	60	51	6	40 — 66	41 — 65
Alveolar dead space ventilation	% $V_{A_{V_D}}$	% of total V_E	60	6	2	2 — 9	3 — 9

* BTPS = Volume corrected to body temperature and pressure and saturation with water vapor.
† STPD = Volume corrected to standard conditions (273° K, 760 mm Hg, dry).

TABLE II.—*Values Obtained by Single Measurements on 10 Male and 10 Female Beagle Dogs Aged 7 to 10 Years*

Parameter	Symbol	Units	Mean	Standard Deviation	Range
Body weight	wt	kg	12.7‡	2.6	7.9 — 18.3
Respiratory frequency (room air)	f	breaths/min	22	7	11 — 36
Tidal volume (room air)	V_t	ml BTPS*	197	67	123 —412
Minute volume (room air)	V_E	liters BTPS/min	4.18	1.64	1.74 — 7.17
Respiratory frequency (100% O_2)	f	breaths/min	18	6	11 — 29
Tidal volume (100% O_2)	V_t	ml BTPS	204	45	158 —298
Minute volume (100% O_2)	V_E	liters BTPS/min	3.47	0.75	2.09 — 4.67
Dynamic pulmonary compliance	$C_{L\ dyn}$	ml/cm H_2O	45‡	24	25 —109
Dynamic pulmonary resistance	$R_{L\ dyn}$	cmH_2O/liters per sec	17‡	11	3 — 40
Functional residual capacity	FRC	ml BTPS	440	129	260 —816
Lung clearance index	LCI	liters V_E/liter FRC	23	5	11 — 32
CO diffusing capacity	$D_{L_{CO}}$	ml/min/mm Hg	8.3	3.1	3.8 — 13.8
Fractional uptake of CO	$F_{U_{CO}}$	decimal fraction	0.477	0.068	0.374— 0.611
Alveolar O_2 tension	PA_{O_2}	mm Hg	80	8	64 — 91
Arterial O_2 tension	Pa_{O_2}	mm Hg	70‡	10	51 — 85
Alevolar-arterial O_2 gradient	$PA\text{-}a_{O_2}$	mm Hg	10‡	5	2 — 21
Arterial CO_2 tension	$Paco_2$	mm Hg	42	6	33 — 58
Alveolar CO_2 tension	$PAco_2$	mm Hg	37	5	29 — 47
Arterial-alveolar CO_2 gradient	$PA\text{-}Aco_2$	mm Hg	5	4	1 — 17
Arterial pH	pH	mm Hg	7.37	0.03	7.29 — 7.41
O_2 saturation of arterial hemoglobin	So_2	%	88‡	5	76 — 94
O_2 consumption	Vo_2	ml STPD†/min	94	35	55 —207
CO_2 elimination	Vco_2	ml STPD/min	95	38	52 —201
Respiratory exchange ratio	RER	Vco_2/Vo_2	1.00	0.11	0.83 — 1.29
Specific ventilation	V_EVo_2	mlV_E/mlVo_2	45‡	10	29 — 63
Alveolar ventilation	% V_A	% of total V_E	54‡	4	46 — 62
Effective ventilation	% V_{eff}	% of total V_E	48	6	36 — 60
Alveolar dead space ventilation	% $V_{A_{V_D}}$	% of total V_E	6	3	2 — 15

* BTPS = Volume corrected to body temperature and pressure and saturation with water vapor.
† STPD = Volume corrected to standard conditions (273°K, 760 mm Hg, dry).
‡ Indicates parameters with means significantly different from identical measurements on younger dogs as shown in Table I.

TABLE III.—*Values at Rest and During Treadmill Exercise Obtained by Duplicate Measurements on 7 Male and 6 Female Beagle Dogs Aged 12–14 Months*

Parameter	Units	Mean	Standard Deviation	Range
Speed = 0.0 mph				
Respiratory frequency	breaths/min	12	4	4 — 20
Tidal volume	ml BTPS*	506	116	309 —677
Minute volume	liters BTPS/min	5.35	1.20	3.22— 6.69
O_2 consumption	ml STPD†/min	115	21	89 —152
CO_2 elimination	ml STPD/min	97	21	66 —139
Respiratory exchange ratio	Vco_2/Vo_2	0.84	0.05	0.74— 0.93
Specific ventilation	ml V_E/ml Vo_2	47	8	36 — 62
Speed = 3.0 mph				
Respiratory frequency		95	57	28 — 2.0
Tidal volume		329	158	168 —675
Minute volume		24.82	6.44	15.35— 39.63
O_2 consumption		440	83	304 —573
CO_2 elimination		380	82	282 —550
Respiratory exchange ratio		0.86	0.05	0.76— 0.96
Specific ventilation		57	13	35 — 78
Speed = 4.5 mph				
Respiratory frequency		126	30	85 —154
Tidal volume		302	70	210 —436
Minute volume		35.17	7.68	20.54— 44.25
O_2 consumption		555	120	366 —688
CO_2 elimination		466	100	289 —577
Respiratory exchange ratio		0.83	0.05	0.75— 0.89
Specific ventilation		65	14	39 — 91

* BTPS = Volume corrected to body temperature and pressure and saturation with water vapor.
† STPD = Volume corrected to standard conditions (273° K, 760 mm Hg, dry).

TABLE IV.—*A comparison of values for selected parameters obtained by similar methods from Beagle dogs and human subjects at approximately the same altitude. Values for Beagles are taken from Table 1. Values for humans were obtained by duplicate measurements performed on 10 normal, adult, male subjects aged 17 to 38 years.**

Parameter	Beagle Dog Mean	Beagle Dog Range	Human Mean	Human Range
Alveolar O_2 tension	83	71–97	85	77–100
Arterial O_2 tension	75	64–90	73	65–83
Alveolar-arterial O_2 gradient	8	1–15	12	3–24
Arterial CO_2 tension	41	31–50	36	32–42
Alveolar CO_2 tension	37	27–45	33	28–39
Arterial-alveolar CO_2 gradient	4	0–7	3	−2–6
Arterial pH	7.38	7.31–7.45	7.45	7.41–7.50
Specific ventilation	40	28–57	34	24–60

* Values provided by Dr. U. C. Luft, Dept. of Physiology, Lovelace Foundation for Medical Education and Research, Albuquerque, N. M.

though the difference from the younger group was not significant at the 95% level. Donevan et al.,[21] using a steady state CO method, reported a lower mean diffusing capacity in persons over 35 than in those under 35. Other authors using various methods, reported similar findings.[22-24]. The finding of a significantly lower pulmonary compliance in the limited number of older dogs tested presents a contrast to changes in the compliance of human lungs reported to occur with aging. The elastic recoil of the lungs is lower in elderly persons than in younger subjects, resulting in an increase in pulmonary compliance with age.[25-26] This apparent species difference is unexplained at this time.

The ease of performing pulmonary function tests on unanesthetized dogs and the usefulness of those tests in appraising pathophysiologic alterations of dog lungs has been demonstrated.[27-30] The techniques described eliminate both the risk of anesthetic procedures and the variable changes in pulmonary function that may be related to anesthesia. The similarities observed between Beagle dog and human pulmonary function and in the changes of certain function parameters with age indicate the usefulness of the Beagle dog as a model for studying a variety of research problems in the fields of pulmonary toxicology, lung aging, and general physiology of the lung.

ACKNOWLEDGMENT

The authors are grateful to Dr. U. C. Luft for his guidance during the development of the procedures described, to Mr. W. C. Nenno, Mrs. G. A. Morrison, and Mrs. P. B. Beckley for conducting the measurements, and to the technical staff who aided in the development and performance of the tests.

REFERENCES

1. SWEANY, S. K., MOSS, W. T., and HADDY, F. J. The effects of chest irradiation on pulmonary function. J. Clin. Invest. 38:587–593, 1959.
2. TEATES, C. D. Effects of unilateral thoracic irradiation on lung function. J. Appl. Phys. 20:628–636, 1965.
3. WOLFE, S. F., BENFIELD, J. R., CRUMMY, A. B., BRIGGS, R. C., and CREE, E. M. Early radiation pneumonitis. Ann. Thorac. Surg. 4:399–411, 1967.
4. SCHREINER, B. F., MICHAELSON, S. M., and YUILE, C. L. The effects of thoracic irradiation upon cardiopulmonary function in the dog. Amer. Rev. Resp. Dis. 99:205–218, 1968.
5. HOOYKAAS, J. A. P., BENFIELD, J. R., WOLFE, S. F., FRENCH, S. W., and CRUMMY, A. B. A physiologic and anatomic study after pulmonary irradiation in dogs. A 52-week report. Dis. Chest 55:123–126, 1969.
6. DUBIN, S. E., and MORRISON, G. A face mask and mouthpiece for respiratory studies in unanesthetized Beagle dogs. J. Appl. Physiol. 27:104–105, 1969.
7. MAUDERLY, J. L., NENNO, W. C., and MORRISON, G. Stocks for holding unanesthetized dogs in the standing position. Lab. Anim. Sci. 21:263–266, 1971.
8. MEAD, J., and WHITTENBERGER, J. L. Physical properties of human lungs measured during spontaneous respiration. J. Appl. Physiol. 5:779–796, 1953.
9. DUBIN, S. E., and WESTCOTT, R. J. Functional residual capacity of normal unanesthetized Beagle dogs. Am. J. Vet. Res. 30:2027–2030, 1969.
10. BECKLAKE, M. R. A new index of the intrapulmonary mixture of inspired air. Thorax 7:111–116, 1952.
11. FOWLER, W. S., CORNISH, E. R., JR., and KETY, S. S. Lung function studies. VIII. Analysis of alveolar ventilation by pulmonary N_2 clearance curves. J. Clin. Invest. 31:40–50, 1952.
12. MAUDERLY, J. L. Steady state carbon monoxide diffusing capacity of unanesthetized Beagle dogs. Am. J. Vet. Res. 33:1485–1491, 1972.
13. PICKRELL, J. A., DUBIN, S. E., and ELLIOTT, J. C. Normal respiratory parameters of unanesthetized Beagle dogs. Lab. Anim. Sci. 21:677–679, 1971.

14. PICKRELL, J. A., and SCHLUTER, S. J. The rapid processing of canine blood gas tension and pH measurements. Am. J. Vet. Res. In press, 1972.
15. LUFT, U. C. and FINKELSTEIN, S. Hypoxia: A clinical-physiological approach. Aerospace Med. 39:106–110, 1968.
16. HERRERA, L. The precision of percentiles in establishing normal limits in medicine. J. Lab. Clin. Med. 52:34–42, 1958.
17. NEERGAARD, K. v., and WIRZ, K. Uber eine Methode zur Messung der Lungenelastizitat am lebenden Menschen, insbesondere beim Emphysem. Z. Klin. Med. 105:35–50, 1927.
18. ROBINSON, S. Experimental studies of physical fitness in relation to age. Arbeitsphysiol. 10:251–323, 1938.
19. LOEW, P. G., and THEWS, G. Die Altersabhangigkeit des arteriellen Sauerstoffdruckes bei der berufstatigen Bevolkerung. Klin. Wschr. 40:1093–1098, 1962.
20. SCHERRER, M., and BIRCHLER, A. Die Altersabhangigkeit des alveolo-arteriellen O_2-Partialdruckgradienten bei Schwerarbeit in Normoxie, Hypoxie, und Hyperoxie. Med. Thorac. 24:99–177, 1967.
21. DONEVAN, R. E., PALMER, W. H., VARVIS, C. J., and BATES, D. V. Influence of age on pulmonary diffusing capacity. J. Appl. Physiol. 14:483–492, 1959.
22. BATES, D. V., and CHRISTIE, R. V. Effects of aging on respiratory function in man. Ciba Foundation Colloq. on Aging, 1:56–68, 1955.
23. OGILVIE, C. M., FORSTER, R. E., BLAKEMORE, W. S., and MORTON, J. W. A standardized breathholding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. J. Clin. Invest. 26:1–17, 1957.
24. MCGRATH, M. W., and THOMSON, M. L. The effect of age, body size and lung volume changes on alveolar-capillary permeability and diffusing capacity in man. J. Physiol. (London) 146:572–582, 1959.
25. FRANK, N. R., MEAD, J., and FERRIS, B. G., JR. The mechanical behavior of the lung in healthy elderly persons. J. Clin. Invest. 36:1680–1687, 1957.
26. PIERCE, J. A., and HOCOTT, J. B. Elastic Properties of the lung in the aged. J. Lab. Clin. Med. 51:63–71, 1958.
27. MCCLELLAN, R. O. BARNES, J. E. BOECKER, B. B., CHIFFELLE, T. L., HOBBS, C. H., JONES, R. K., MAUDERLY, J. L., PICKRELL, J. A., and REDMAN, H. C. Toxicity of beta-emitting radionuclides inhaled in fused clay particles—an experimental approach. In *Proceedings of Conference on Morphology of Experimental Respiratory Carcinogenesis*, Gatlinburg, Tenn., May 13–16, 1970.
28. THOMAS, R. G., MCCLELLAN, R. O., THOMAS, RANDI L., CHIFFELLE, T. L., HOBBS, C. H., JONES, R. K., MAUDERLY, J. L., and PICKRELL, J. A. Metabolism, dosimetry and biological effects of inhaled ^{241}Am in Beagle dogs. To be published in *Proceedings of Biological Implications of the Transuranium Elements Symposium*, Richland, Washington, September 26–29, 1971 and Health Phys., June 1972.
29. HOBB, C. H., BARNES, J. E., MCCLELLAN, R. O., CHIFFELLE, T. L., JONES, R. K., LUNDGREN, D. L., MAUDERLY, J. L., PICKRELL, J. A., and RYPKA, E. W. Toxicity in the dog of inhaled ^{90}Y in fused clay particles: Early biological effects. Radiat. Res. 49: 430–460, 1972.
30. MUGGENBURG, B. A., MAUDERLY, J. L., PICKRELL, J. A., CHIFFELLE, T. L., JONES, R. K., MCCLELLAN, R. O., PFLEGER, R. C., and LUFT, U. C. Pathophysiological sequelae of bronchopulmonary lavage. Am. Rev. Resp. Dis. 106:219–232, 1972.

DISCUSSION

W. EHRLICH, Johns Hopkins, Baltimore, Maryland: I wanted to ask you whether you had to remove the mask after the dog ran, or how did the dog handle temperature regulation? It is known that the only place they can sweat or get up air vapor is the tongue.

DR. MAUDERLY: Right. Well, in the first place, remember that I mentioned that we're inspiring dry room air so the elimination of moisture is facilitated in that way. The mask is designed so that the dog can breathe open mouthed. They can pant, they can breathe with any pattern they want. The speeds that we selected were partially selected for these reasons. Above four and a half miles an hour we found the dogs breathing with a very rapid, shallow panting pattern presumably because of temperature and moisture regulation. Three miles an hour was simply chosen as an intermediate speed. Also, four and a half miles an hour was the speed which dogs with significant pulmonary dysfunction in our studies could maintain. But the mask is constructed so they can breathe open mouthed. There's plenty of room around the muzzle. They're on the treadmill actually running for perhaps fifteen minutes. The treadmill is level and does not represent a maximal exercise. We've done some blood lactate measurements on the dogs immediately after they come off the treadmill and we've found no consistent increase in blood lactate so it's a very mild exercise for the animals.

R. A. REDDING, Brown University, Providence, Rhode Island: Three questions. One, is rectal temperature measured during your test

procedures? I believe sometimes with muzzles like this the temperature may increase in animals during this sort of procedure. Secondly, could compliance with age be a frequency dependent change, perhaps because of small air wave changes? Or, the other thing that I've heard about in beagles, is some sort of spontaneous interstitial fibrosis with age. Is this a possibility? Has this been looked at in any way as a cause of the change in compliance?

DR. MAUDERLY: We measure body temperature on each dog during the arterial bleeding procedure, not during the other procedures. We have measured the temperature in the series of dogs several months ago during the other procedures. We found that the mean body temperature during our testing procedures is 102° F. Now, I'm not talking about the exercise procedures, but the other procedures. This doesn't represent a very large increase over what you'd expect for a normal body temperature. The dogs are very calm. The dogs that do show increases in temperature would be those which became excited, so forth, during the test, and these would be the dogs which would most likely have their tests repeated anyway, because of test values received. So it hasn't been a problem for us. We make an effort to maintain the laboratory at a fairly even temperature, about 22° C, but we don't control it as closely as some people have. I don't really feel that body temperature is a very big factor. Now, where it affects the volume that we measure BTPS for our ventilation measurements, and where it affects the alveolar moisture or vapor pressure of water for our alveolar arterial gradients, we do measure the temperature, because this is done during the arterial sampling procedures. So, these are corrected.

Now in relation to the changes found in older dogs, I'm sure that from the few radiographs that we've seen the older dogs, that we do get some fibrotic changes in the lungs. We are just beginning. We've been involved in a very extensive program of placing young adult dogs on these toxicological studies and we're just now getting to the point where we're looking at large numbers of older dogs, and so I'd be much better prepared to answer your question in a few months. But we do see this reduction in compliance. I can't explain exactly why it occurs at this point, but it is a very consistent reduction in compliance. The frequencies observed on the younger and older age groups were not significantly different. Now I haven't done extensive studies of frequency and compliance in individual dogs, but we do measure this over a period of several breaths and I think the values we obtain are probably relatively accurate.

K. SAGAWA, Johns Hopkins, Baltimore, Maryland: Was the beagle particularly good from a behavioral viewpoint? Was that why you chose the beagle?

DR. MAUDERLY: Well, this is the only dog we've worked with. We're working in a closed colony. We have about fifteen hundred beagles. We have our own breeding program located out of town. It's a closed colony so we haven't had an opportunity to work with other breeds or with mongrel dogs. We do find that beagles are very nice for these sorts of studies. I would expect that we would have more problems if we were using pound dogs, or something like that. The beagle, as you all know, is a very even tempered animal.

DR. SAGAWA: Was there any effect of repeated tests on accumulated test results?

DR. MAUDERLY: No, we have not found any as of yet.

R. L. HAMLIN, Ohio State University, Columbus: Have you tried to correlate any of the dynamic compliance measurements with static or quadristatic compliances in the same dogs, anesthetized, to see if the changes with age are not frequency dependent?

DR. MAUDERLY: Well I would have to say no and qualify it this way; so far, as I said, we've been very busy with launching tremendous numbers of dogs on these studies and we've been somewhat restricted in the other projects we would like to work with.

One thing we'd like to do, and intend to do, is to compare the effect of anesthesia with the effect of different methods of measurement. Now I think those of you that are familiar with pulmonary function measurements are well aware of the fact that the results you obtain can be different, depending upon what technique you use. The same is true of the question of whether you're going to work with anesthetized or con-

scious dogs. The results are quite possibly different. We haven't elucidated all these differences at this point. We have simply chosen the model that we can work with for the reasons that I gave, and have done a fairly extensive job of establishing our own values for our own techniques in this model. Now there's nothing I'd like better than to be able to make some of these comparisons and I'm sure that we will, in the future.

P. L. LUNA, Armour Pharmaceuticals, Kankakee, Illinois: Have you had any problems with the saliva from these dogs when you exercise them?

DR. MAUDERLY: No we have not. And I don't know whether this is because of the relatively short time that they're on the treadmill or whether it is because they are inspiring a dry gas. Of course, Albuquerque has a very low mean humidity and this may have something to do with it. There usually is moisture in the mask, but as I said, the mask is relatively square on the front and about 4 inches in depth and eliptical in shape. Therefore, any moisture that might collect, a few cc's, would be down at the bottom and would not interfere with gas collections. But it has not been a problem for us to this point.

G. E. BISGARD, University of Wisconsin, Madison: What is your experience with the mass spectrometer? I've had people give me conflicting reports on having considerable difficulties keeping them in working order and so forth. How have you done with that? In our studies with calves, we've had problems getting good end tidal gas samples. We don't feel that we can get real good samples because of the sometimes fast respiratory rates, and we wonder about your experience with dogs in this area.

DR. MAUDERLY: Well, in relation to your first question about mass spectrometry, I think that's a little bit like marriage, in which you have great expectations and then for the first short period of time after you achieve this status, you have great doubts and then you settle down to where you are used to the equipment and you become a pretty handy repairman in the laboratory. Now, as you saw, we use the SRI mass spectrometer which has fixed collectors. It is a very simple machine. It's a very nice machine for laboratory work, easy for technicians to operate, and I'd say that we've had very good luck with it. Now before the advent of our mass spectrometer we were using individual analyzers of different types for the different gases and I think those of you in the audience that saw the lab at that stage can see the improvement. It has eliminated a tremendous problem in continuous calibration and so forth. I would say that our experience has been very good, although it's not optimal at this point.

W. J. MOORMAN, National Institute of Occupational Safety and Health, Cincinnati, Ohio: What is the volume of the dead space in the mask that you use and do you think that this effects ventilation? My second question is: how reliable are your esophageal pressure tracings for the mechanics? So you use any kind of anesthesia or local anesthesia, or do you have esophageal comes? Any problems with this?

DR. MAUDERLY: Well, yes, of course, there are problems in any studies of this type and here again probably the only saving grace is that we're making these measurements over a period of several breaths and therefore we can recognize changes in the hysteresis loops that we see, and if we see something widely occurring, we can wait or calm the dog or try again. We use no local anesthesia or anything in placing the catheters. The dogs take these very well. I am sure that there are some changes and I think when you see the data in the manuscript you'll recognize that we have fairly substantial standard deviations in our compliance measurements. We feel that the pressure fluctuations that we get are relatively reliable. Those static tests that we've done with the catheters and so forth have indicated this. We've noted in our different studies, where we've applied these tests, that the compliance measurements we've obtained have shown very nice changes comparable to those performed by other methods. All I can say at this point, is that it doesn't seem to be a problem. There are definitely variations that occur. In comparing the dog with baseline testing prior to entry on a study, if his measurements are outside certain limits, we'll retest him, and I think quite often the reason for these

differences is esophageal tone or other variations.

We've compared values, say, for carbon dioxide obtained with our end tidal sampler and the breath by breath peak method, and they're very close. Now, we feel that we can get legitimate end tidal samples to frequencies up around 50. In our dogs with radiation damage, of course, the frequency continues to climb until death. Certainly we place no reliance on frequencies of 80 to 100, and in fact, stop our measurements of diffusing capacities when frequencies reach that high. However, the plunger, that I noted in the pressure chamber, allows the collection of a preset volume. Therefore, if we have an idea of the tidal volume of the dog (most of our dogs are about the same size) and know the frequency, we can guess fairly closely the tidal volume and we usually set the collection at about 10%; so you can see we're well up on the alveolar plateau. End tidal measurements or alveolar measurements are a big bucket of worms in pulmonary physiology. Here again, I think you have to set your conditions, and decide how you're going to do it in your laboratory. It may or may not compare closely with what someone else is doing, but you're all familiar with the problems of the definition of true alveolar gas, mean alveolar gas and so forth. So we've set our conditions and we're making our measurements. We feel that we get good samples with it. They compare very favorably to those obtained in humans.

H. CALDERWOOD, University of Florida, Gainsville: How reproducible are your steady state diffusing capacities within the same dogs? We're doing pulmonary function tests on anesthetized beagles and had to give up the steady state and go to the single breath to get reproducibility.

DR. MAUDERLY: Well this is very true. The reproducibility is not good. There is quite a high variation. The variation within a dog on sequential measurements is lower than it is within the colony. Of course, here when I mention colony, I'm not talking about a large range of sizes, as you could see with pound dogs, but with a fairly narrow range of sizes that we see in our beagles. The fluctuation of this value within an individual dog is less, but it is still quite large. We place very little reliance on a single measurement. But here again performing the measurements in the conscious state, we have these variations and therefore we rely on numbers, and our tests don't take very long. They're relatively innocuous and we can perform them repeatedly with very little trouble. So we rely on two or three tests on the same animal and come up with a mean value. But there is a considerable variation by method and I'm sure more variation than others' have seen with single breath method.

C. G. LA FARGE, Children's Hospital, Boston, Mass.: In this very nifty animal model that you've shown so beautifully this morning, are there important gravitational effects on ventilation and profusion? I notice that you do some of your experiments in what might be called a normal dog position and others in the inverse?

DR. MAUDERLY: This is very true, and it's a question which I feel we'll have to address very soon. We have not done specific experiments designed to answer that question. I'm sure that there must be some gravitational effects as there are in humans. I think the effects are quite possibly less because of the rather horizontal axis of the dog's lung and the relationship of the lung to the heart. So, therefore, if you turn the dog on its back, you really aren't changing the position of the heart and the lungs too much. Now, quite possibly some of the differences we see here in pH and in the carbon dioxide parameters may be related to the fact that they're restrained on their backs, but we have not specifically addressed that question.

A. AZAR, E. I. DuPont, Newark, Delaware: During the course of your toxicity testing, have you had an opportunity to use this technique where you've exposed animals to compounds known to effect pulmonary function; and if you have, would you comment on the sensitivity of the method in detecting changes where animals have been exposed to, say, a TDI or a compound known to effect pulmonary function?

DR. MAUDERLY: Yes, as a matter of fact, most of our work is with radioactive aerosols. The effects are very similar to what you find from external radiation. We have found that the occurrence of lung damage can be picked up

with these function tests prior to the point at which it can be picked up clinically. We worked with this very intensely. Now, we haven't taken, for instance, isoproterenol, or some other drug, and injected it; measuring changes. Our studies have been of a different nature, a different design. But we have seen very nice directional changes. We have seen the onset of these directional changes to occur on pulmonary function testing before they were obvious with other methods. And, we are working on some other techniques which I did not mention today, which are more sensitive. We're working for a sensitive indicator, as well as simply a pathogenesis of the disease. But it has worked very well for us. I don't have any data in the manuscript, as such, on pulmonary function changes related to a disease condition. It wasn't within the scope. But I do have a tremendous volume of that sort of information, and we do see very nice directional changes.

CHAIRMAN: You don't think then that the dog's normal pH is 7.3 arterial, as you had a normal range?

DR. MAUDERLY: Well, this is the normal that we found on sixty dogs, and therefore it is our normal value. Now, again when you develop tests like this you have to set forth your conditions and then establish your base line or normal values based on those conditions. Now, the dog resting back in the kennel may have a slightly different pH. This is the normal pH that we found in our laboratory. And other compilations of the data that we've made over the past few years give slightly different means. It may be 7.38, or 7.4, or 7.375. But it's very close to this. I think this is a good figure.

CHANGES IN LEFT VENTRICULAR HEMODYNAMICS AND DIMENSIONS DURING REJECTION OF THE ORTHOTOPICALLY TRANSPLANTED CANINE HEART

E. B. Stinson, P. L. Tecklenberg, J. F. Hollingsworth and K. W. Jones*

At the time of orthotopic cardiac transplantation in fourteen dogs an indwelling arterial pressure catheter was inserted into the recipient aorta and an electromagnetic flow transducer placed around the donor aorta. Serial electrocardiographic and hemodynamic studies were performed immediately postoperatively and then twice daily until rejection. Immunosuppressive drugs were not given. In an additional four dogs a miniature solid-state pressure transducer was placed within the transplanted left ventricle (LV) and endocardial piezoelectric crystals across the maximum transverse LV diameter; these animals were studied immediately and then daily until death.

Immediately postoperatively stroke volume and cardiac output were severely depressed (6.0 ml and 41.6 ml/kg/min), but then rose progressively over the next 48 to 72 hours by 93 and 76% respectively; heart rate declined during this time from 133 to 102 beats/min. Arterial blood pressure showed no significant changes from the time of operation until shortly before death. Peak LV systolic pressure and dP/dt were stable during the early postoperative period; calculated ejection fraction and peak circumferential fiber shortening rate increased over the first 24 hours from 0.55 to 0.64 and from 2.6 to 2.9 circs/sec, respectively, and thereafter remained stable until rejection.

Average survival was 6.5 days (± .6 S.E.). At the time of onset of acute rejection, as defined by a progressive decrease in electrocardiographic voltage (one to four days before death), there were no consistent detectable changes in hemodynamics or LV dimensions. Subsequently, in the preterminal period, stroke volume and cardiac output declined to 5.9 ml and 34.5 ml/kg/min, respectively. Ejection fraction and peak V_{CF} diminished to 0.56 and 1.9 circs/sec, respectively. Peak LV and arterial pressure and LV dP/dt were well-maintained until terminal collapse of the animals. These studies indicate that at rest the pumping performance of the transplanted heart is not detectably impaired until rejection injury is advanced, and thus hemodynamic observations offer little aid in the early diagnosis of rejection.

INTRODUCTION

Although clinical observations,[1] as well as isolated hemodynamic findings,[2] after cardiac transplantation in man suggest that ventricular performance deteriorates progressively during the course of acute graft rejection, serial studies of cardiac function during this period have not been presented in detail. In the investigation described below, changes in hemodynamic status and alterations in left ventricular internal transverse diameter were studied in dogs during acute rejection of the orthotopically transplanted heart.

METHODS

Orthotopic cardiac transplantation was performed in 14 dogs by the method of Lower and Shumway.[3,8-11] Donor-recipient pairs were matched only for approximate size. No drugs beside antibiotics (penicillin and streptomycin), and in some cases a single intraoperative dose of digoxin, were given at the time of operation or thereafter. Donor hearts were electrically paced via atrial leads at 140–160 beats per minute for 24–48 hours postoperatively except during periods of study.

In 10 animals a snug-fitting electromagnetic flow transducer was placed at the time of transplantation around the aorta or pulmonary artery of the graft; when the aorta was utilized, a thin mesh of Dacron was placed beneath the proximal edge of the flow transducer to prevent erosion (this did not significantly affect calibration). In addition, a wide-bore polyvinyl catheter (10F) was inserted into the recipient's left carotid or subclavian artery to the level of the aorta. The flow transducers were calibrated with saline or blood before and after implantation, and paired calibrations were essentially identical.

In four additional animals instruments were implanted at the time of operation to permit measurement of left ventricular pressure and

* Clinic of Surgery, National Heart & Lung Institute Bethesda, Maryland 20014.

internal transverse diameter. Ventricular pressure was measured with a miniature solid-state pressure transducer* (7 mm diameter) precalibrated in-vitro at 38°C. This was inserted into the left ventricular cavity through the apical dimple after excision of the donor heart. The gauge was positioned approximately 5–8 mm inside the endocardial surface to avoid distortion of the pressure waveform by muscular entrapment during systole, and was fixed in place at the epicardium by a pursestring suture. Static calibration of the gauges against a mercury column was linear from zero to 300 mm Hg (within one percent) without hysteresis. Frequency response was limited by the recorder and was linear to 80 cps (± 5%). Zero stability in pre- and postimplantation calibrations was not found to be reliable, and thus measurement of absolute pressures was not attempted. The rate of left ventricular pressure change was obtained with an active differentiator having a frequency response linear to 80 cps.

Instantaneous, continuous measurements of internal transverse left ventricular diameter were obtained by ultrasound techniques described elsewhere.[4] Briefly, two 5 mm diameter discs of lead titante-zirconate crystal, mounted in lucite housings and fitted with convex lucite lenses, were implanted on opposite endocardial surfaces of the left ventricle across the maximum transverse diameter, in an approximately anteroposterior orientation. Lead wires exited through the ventricular wall of the site of implantation, and were exteriorized, along with other leads, at the back of the animal's neck. The transmitting crystal was pulsed for one microsecond at a 5.6 khz repetition rate. The received signal from the opposite crystal, after rectification and amplification, was detected by a tracking gate similar to that used in radar systems.[5] The analog output of the tracking gate was proportional to distance between the crystals (transmitting time) and was precalibrated *in vitro*. The rate of change of the ventricular diameter thus measured was obtained by passive differentiation of the tracking gate output after low pass filtering. The phase lag through tracker and filter combined was linear to 20 cps.

* Model P-22, Konigsberg Instruments, Pasadena, California.

All animals were studied in the intact, awake state at rest, lying on the right side, within six hours postoperatively, and thereafter at least once daily until death due to acute graft rejection. Arterial blood gas analyses performed in the majority of dogs during the initial postoperative study, while they were spontaneously breathing air, showed average pH 7.41 (± .02 S. E.), average pO_2 83.6 mm Hg (± 2.6 S.E.), average oxygen saturation 95.9% (± .4 S.E.), average pCO_2 29.8 mm Hg (± 1.9 S.E.), and average bicarbonate concentration 18.1 mEq. per liter (± 1.3 S.E.).

During each study a 3-lead, standardized electrocardiogram (limb leads I, II, III), the flow signal,* aortic blood pressure** (referenced to midchest level), left ventricular pressure and dp/dt, and the left ventricular diameter signal as well as its first derivative, were recorded on either a Beckman type R Dynograph or Brush 480 recorder and on magnetic tape.*** Heart rate, stroke volume, cardiac output, mean aortic blood pressure, and peripheral vascular resistance were calculated off-line with the aid of a digital computer. Peak-to-peak QRS voltage in lead II (normally conducted complexes) and cardiac rhythm were ascertained by inspection.

End-diastolic diameter (Dd) was determined at the onset of isovolumic contraction, and end-systolic diameter (Ds) was taken as the minimum systolic diameter. These measurements were averaged over several cardiac cycles selected during expiratory pauses. The difference between end-diastolic and end-systolic diameters, divided by end-diastolic diameter, represented the percent systolic shortening (Dd-Ds)/Dd. The diameter at the end of early, rapid diastolic filling was designated Dr, and the ratio Dr-Ds/Dd-Ds defined as the percent of rapid filling.

The instantaneous rate of left ventricular diameter shortening (dD/dt) was determined directly from the differentiated diameter signal. Maximum systolic—dD/dt was divided by the corresponding instantaneous diameter to obtain

* Biotronex 610 Pulsed-logic Flowmeter, Biotronex Lab, Silver Spring, Md.

** Statham P23db, Statham Instruments, Hato Rey, Puerto Rico.

*** Ampex FR 1300, Ampex Corp., Redwood City, California.

peak circumferential fiber shortening rate in circumferences per second (V_{CF}).

To facilitate comparisons between animals, the results of hemodynamic studies at each daily interval during the first 72 hours after transplantation and during the last 72 hours of life in the first group of 10 animals were averaged. The initial postoperative electrocardiogram in all animals was taken as a baseline "control" to which subsequent electrocardiograms were compared. Records from the second group of four animals were selected from the immediate postoperative period, the third postoperative day (chosen because the majority of dogs in the first group demonstrated maximum hemodynamic recovery at this time), and from the time of diagnosed acute rejection (within 24 hours of terminal graft failure).

Student's t-test was used to compare grouped observations, assigning a two-tailed significance level to the P value.

RESULTS

All dogs died of acute graft rejection four to 12 days postoperatively (mean 6.5 days ± 0.6 S.E.). Histologic findings characteristic of advanced first-set rejection[6] were present.

Initial Hemodynamics

Immediately postoperatively, the average heart rate was 133 beats per minute (± 7.1 S.E.); over the ensuing 72 hours, mean heart rate decreased gradually to 102 beats per minute (± 8.8 S.E.). Mean cardiac output was initially quite low, 41.6 ml/kg/minute (± 6.4 S.E.); it then rose steadily over the next 48 to 72 hours to reach a relatively stable plateau value of 78.6 ml/kg/min (± 6.4 S.E.). Similarly, stroke volume was markedly depressed immediately after transplantation with a mean value of 6.0 ml (± .8 S.E.), but rose over the next two to three days to 11.6 ml (± 1.1 S.E.). In contrast to the early postoperative increases in stroke volume and cardiac output, mean arterial pressure showed no significant variations from the time of transplantation until late in the courses of the animals (average approximately 92 mm Hg). Mean peak left ventricular dp/dt was 2707 mm Hg/second (± 215 S.E.) immediately after operation, and remained near this level throughout the first three days (Figure 1).

Left ventricular internal transverse diameter at end-diastole ranged from 16.5 to 34 mm with an average of 24.9 mm immediately postoperatively. Little change in end-diastolic diameter occurred throughout the first three postoperative days; heart rates in these animals were relatively stable during this interval. The average maximum rate of systolic diameter shortening (-dD/dt) rose from 55.8 mm/second initially to 62.5 mm/second ($p>.05$) on the third postoperative day (Figure 1). Normalized circumferential fiber shortening rate increased from 2.6 to 2.9 circumferences/second ($p>.05$) (Figure 1). Percent systolic shortening of the transverse diameter rose in three of the four dogs throughout the defined recovery period, from an average 24.8% to 28% ($p>.05$) (Figure 1).

In one animal systolic -dD/dt and V_{CF} were also determined at the point of peak total wall tension, which was derived as the instantaneous product of left ventricular pressure and radius divided by two (PR)/2. Although the absolute values for tension obtained in this manner were unreliable because of the limitations of pressure measurement, identification of relative changes in the tension state permitted an estimate of contractile element velocity during ejection (VCE), at the point of maximum tension.[7] Values thus derived showed an approximate parallel relationship to peak -dD/dt and V_{CF}, demonstrating an increase from 41 mm/second and 1.8 circs/second, respectively, at the time of transplantation to 67 mm/second and 2.6 circs/second, respectively, on the third postoperative day.

Acute Rejection

All animals recovered to the point of normal or nearly normal ordinary activity over the first three to four postoperative days, and remained so until one to two days prior to death. Terminally, signs of low cardiac output were present, and auscultation consistently revealed a diastolic gallop heart sound. Only rarely, however, were abnormal breath sounds audible.

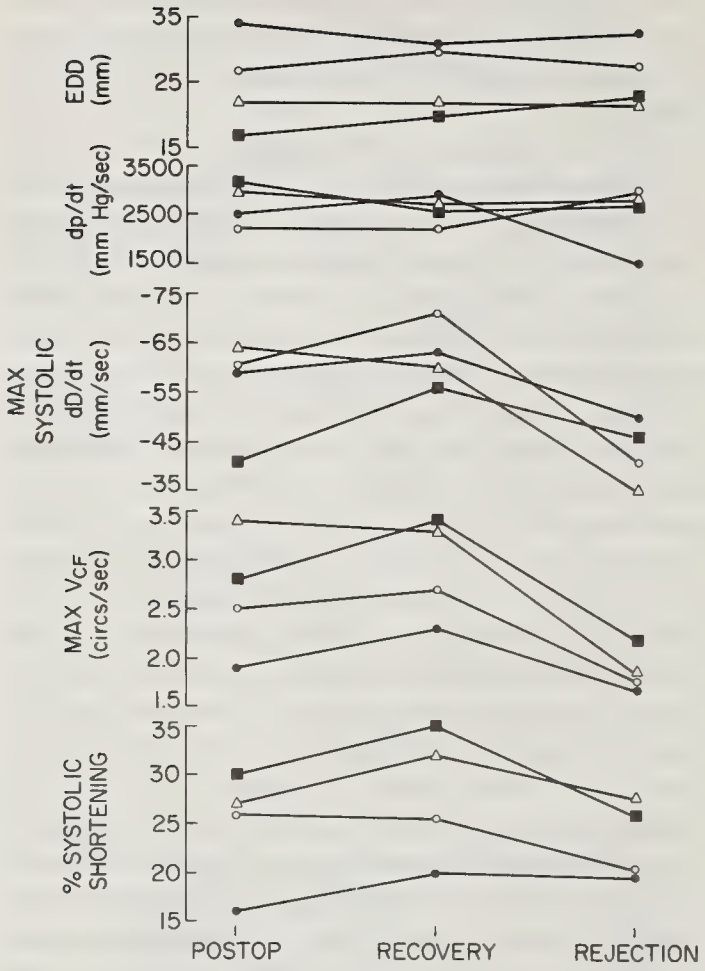


FIGURE 1.—Changes in left ventricular internal transverse diameter and diameter shortening in four orthotopic cardiac grafts immediately after transplantation, on the third postoperative day (recovery), and during acute rejection.

Mean QRS voltage in lead II as a function of the initial postoperative recording showed a progressive decline during the course of acute rejection (Figure 2). A decrease in voltage appeared one to four days prior to death in individual dogs, but the decline in mean values attained statistical significance only one day before death.

Average heart rate, excluding one dog with transient atrial fibrillation, declined only slightly during rejection ($p > .05$). Stroke output, however, diminished significantly ($p < .05$) during advanced rejection (Figure 2), and terminally reached a mean value of 5.9 ml (± 1.3 S.E.), or approximately 50% of the mean stroke volume previously recorded at the point of maximum hemodynamic recovery after transplantation. The progressive decline in stroke volume accounted almost entirely for the fall in cardiac output with rejection (Figure 2); mean cardiac output decreased late in rejection to 35 ml/kg/minute (± 3.9 S.E.) or 45% of the previously achieved average highest cardiac output for all dogs ($p < .05$). Mean arterial blood pressure remained relatively stable, despite the deterioration of cardiac output, until terminal collapse of the animals (Figure 2). Similarly, peak left ventricular dp/dt was well maintained during rejection in three of the four dogs studied; in the fourth subject, peak dp/dt, measured immediately before death, was decreased in comparison to previous recordings (Figure 1).

End-diastolic diameter showed only slight variation during rejection in the four animals studied (Figure 1). The small changes which did occur were inversely related to individual variations in heart rate, although mean heart rate during rejection was not significantly dif-

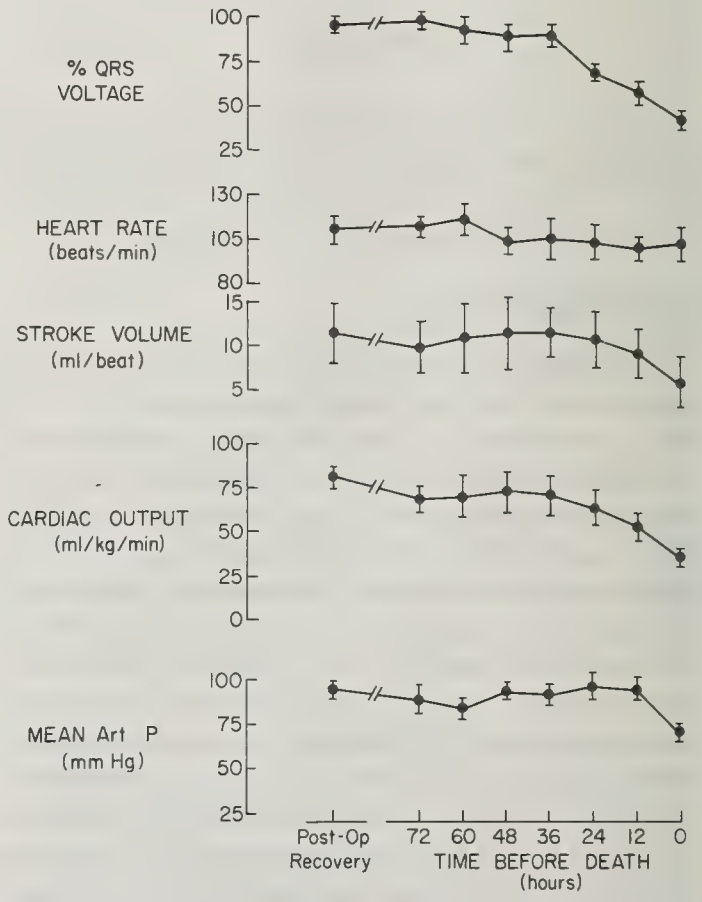


FIGURE 2.—Mean values ± S.E. for QRS voltage in lead II (as percent of initial recording) and for hemodynamics in ten dogs at the time of maximum hemodynamic recovery (second or third postoperative day) and during acute rejection.

ferent from that present at the time of postoperative recovery.

Both peak systolic rate of diameter shortening (-dD/dt) and normalized circumferential shortening rate (V_{CF}) decreased significantly ($p < .05$) with acute rejection (to 43 mm/second and 1.9 circs/second, respectively) (Figure 1). Percent systolic shortening of the transverse diameter also declined, from a mean value of 28% recorded at the time of recovery to 23.5% ($p < .05$) (Figure 1).

Maximum systolic -dD/dt and V_{CF} determined at the point of peak wall tension in one animal decreased during graft rejection to final measured values of 30 mm/second and 1.1 circs/second, respectively.

In all four grafts in which transverse left ventricular diameter was serially studied striking changes in the pattern of diastolic filling developed during rejection. The early, rapid phase of diastolic filling, which at the time of postoperative recovery accounted for 60 to 70% of the total diastolic increment in diameter, accounted for a progressively increasing proportion of total diastolic filling with advancing rejection ($p < .01$) (Figure 3). In other words, during late rejection little or no increase in left ventricular diameter occurred after the rapid filling phase, despite the persistence of donor atrial systole. The resulting records of left ventricular diameter were characteristically "flat-topped." Representative serial recordings of left ventricular diameter throughout the postoperative course of one animal are shown in Figure 4. Concurrently, the average rate of diameter lengthening during the rapid filling phase of diastole decreased significantly (Figure 3). Mean rapid filling dD/dt for the four animals decreased from 71.3 mm/second at the time of postoperative recovery to 54 mm/second during rejection ($p < .01$).

DISCUSSION

These studies indicate that in the unmodified canine recipient hemodynamic function of an orthotopic cardiac graft is relatively well-maintained at rest until late in the course of immunologic rejection. Detectable impairment of pumping performance developed only during the terminal 24 to 36 hours. In contrast, histologic observations have demonstrated a progressive immune response appearing as early as 24 to 48 hours after transplantation.[6] The earliest such changes consist of mononuclear cell margination, endothelial cell damage, edema formation, and then progressive perivascular and interstitial mononuclear cell infiltration.[6] It is apparent from the present studies that in the initial stages of rejection these changes are not expressed in demonstrable deterioration of hemodynamic or mechanical function of the transplanted left ventricle. Late in the course of rejection, i.e., 24 to 48 hours prior to terminal failure of the graft, morphologic lesions include widespread foci of myocyte necrosis, and, in some cases, areas of frank myocardial infarction involving especially the left ventricular subendocardial region and papillary muscles. Also at this stage, edema of the ventricular walls is strikingly severe, and the ventricular myocardium is grossly firm, rubbery, and dusky-red.

During this interval, progressive diminution of cardiac output, due almost entirely to in-

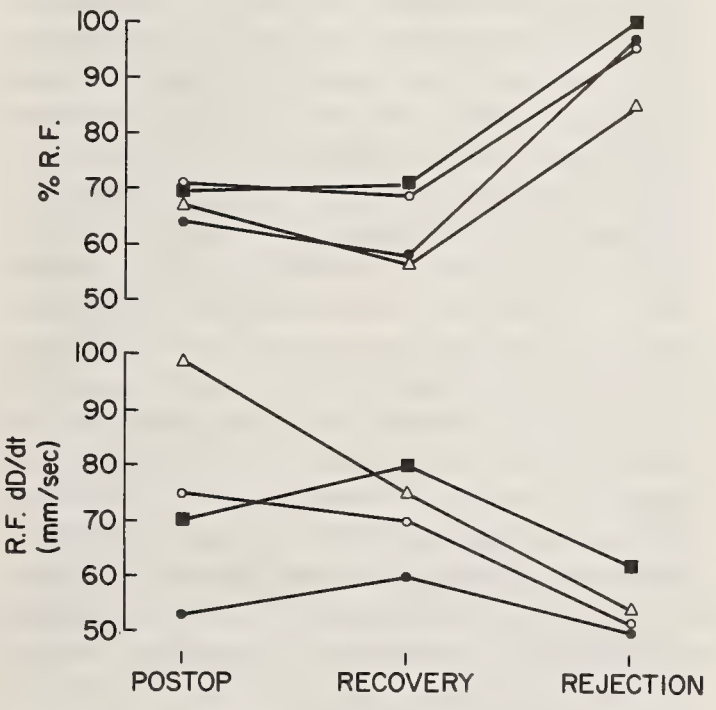


FIGURE 3.—Changes in the percent of rapid diastolic filling (% R.F.) (see text) and average dD/dt during rapid filling in four grafts (time periods same as in Figure 1).

creasing restriction of stroke volume, develops and eventually results in death of the host. Uncommonly, a fatal arrhythmia (most often asystole, not ventricular fibrillation) is the terminal event. It is important to note that parallel augmentation of peripheral vascular resistance in the recipient results in relatively stable preservation of arterial blood pressure (and thus, parenthetically, in conjunction with the available data on ventricular diameter, maintenance of left ventricular afterload) until terminal collapse.

Of the primary determinants of cardiac output, i.e., ventricular preload, afterload, heart rate, and contractile state, it is the latter which would appear to be the critically limiting factor during advanced rejection. The small changes which did occur in end-diastolic fiber length (diameter) were directionally opposite to those which would tend to decrease stroke volume, according to the Frank-Starling principle. However, these changes were sufficiently slight as to not significantly increase ventricular afterload. Heart rate changes were generally minimal.

The observed alterations in maximum systolic -dD/dt, V_{CF}, V_{CE}, and percent systolic shortening of diameter (and thus by extrapolation, ejection fraction), in the absence of significant variations of preload, afterload, heart rate, and direct neural stimulation (denervated heart), suggest a decay in contractility. Several previously demonstrated pathologic findings, in addition to actual myocyte necrosis, are available for correlation with this conclusion. For example, Kosek and coworkers[6] have shown, in cardiac allografts undergoing acute rejection, abnormalities in subcellular structures such as mitochondria, the sarcotubular system, and endoplasmic reticulum. Such changes would be expected to be associated with derangement of electromechanical coupling and energy transfer. At the present time, however, the precise pathophysiologic implications of these morphologic alterations in acute graft rejection are unknown.

In addition to the changes recorded in donor left ventricular systolic performance during acute rejection, significant alterations in the configuration of diastolic filling were also observed. With advancing rejection the rapid, early phase of ventricular diastolic filling accounted for an increasing proportion of the total increment of diameter during diastole. Because of the limited augmentation of diameter during diastasis and atrial systole, the resulting records of left ventricular diameter assumed a "flat-topped" appearance (Figure 4), reminiscent of the "square root" sign of ventricular

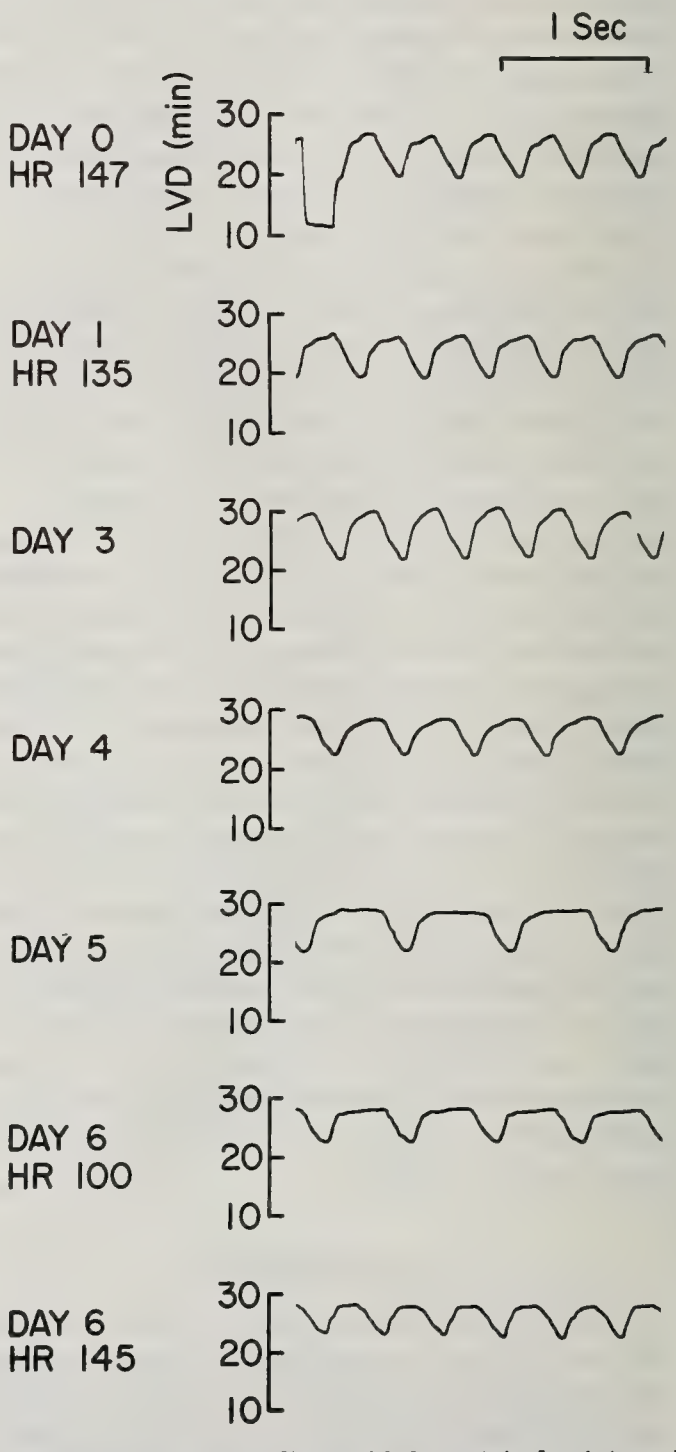


FIGURE 4.—Serial recordings of left ventricular internal transverse diameter (in mm) throughout the postoperative course in one recipient.

pressure curves observed in constrictive or restrictive heart disease. Since these modifications in diastolic filling pattern were independent of concomitant changes in absolute dimensions and heart rate, and were not associated with apparent alterations of ventricular geometry, they may be interpreted as indicative of reduced myocardial compliance. This hypothesis is compatible with the gross and microscopic pathologic findings in acute cardiac graft rejection, namely, firm, rubbery, thickened ventricular walls, severe myocardial edema, vascular congestion, and cellular infiltration. Such features would be expected to produce significant changes in the elastic properties of the ventricular myocardium. It is noteworthy that in two animals distortion of the diastolic diameter waveform developed concurrently with a decrease in QRS voltage, before the onset of significant hemodynamic deterioration.

SUMMARY

These studies demonstrate that acute rejection of orthotopically transplanted hearts in untreated dogs is associated with progressive hemodynamic impairment which develops relatively late in the postoperative course and is expressed primarily in the limitation of stroke volume. The results of additional studies of transverse internal left ventricular diameter during rejection suggest that the decline in ventricular performance is due primarily to depression of the graft contractile state. Decreases in electrocardiographic voltage and alterations in the pattern of donor left ventricular diastolic filling generally preceded hemodynamic deterioration.

REFERENCES

1. Stinson, E. B., Dong, E., Jr., Bieber, C. P., Schroder, J. S., and Shumway, N. E. Cardiac transplantation in man. I. Early rejection. J.A.M.A. 207:2233, 1969.
2. Williams, G. M., DePlanque, B., Braham, W. H., and Lower, R. R. Participation of antibodies in acute cardiac-allograft rejection in man. New England J. Med. 281:1145, 1969.
3. Lower, R. R., and Shumway, N. E. Studies on orthotopic transplantation of the canine heart. Surg. Forum *II*:18–20, 1960.
4. Stinson, E. B., Rahmoeller, G., and Tecklenberg, P. L. Measurement of internal left ventricular diameter by tracked ultrasound. In preparation.
5. Schuette, W. H., and Simon, A. L. A new device for recording cardiac motion. Med. Res. Eng. 7:25, 1968.
6. Kosek, J. C., Hurley, E. J., and Lower, R. R. Histopathology of orthotopic canine cardiac homografts. Lab. Invest. 19:97, 1968.
7. Levine, H. J. Muscle mechanics in the in-situ heart. In: *Factors Influencing Myocardial Contractility*. (Eds. R. D. Tranz, F. Kavaler, J. Roberts.) Academic Press, New York, p. 93, 1967.
8. Lower, R. R., Dong, E., Jr., and Shumway, N. E. Long-term survival of cardiac homografts. Surg. 58:110–119, 1965
9. Lower, R. R., Stofer, R. C., and Shumway, N. E. Homovital transplantation of the heart. J. Thorac. and Cardiovasc. Surg. 41:196, 1961.
10. Chartrand, C., Angell, W. W., Dong, E., Jr., and Shumway, N. E. Atrial pacing in the postoperative management of cardiac homotransplantation. Ann. Thorac. Surg. 8:152–160, 1969.
11. Stinson, E. B., Griepp, R. B., Bieber, C. P., and Shumway, N. E. Hemodynamic observations after orthotopic transplantation of the canine heart. J. Thorac. Cardiovasc. Surg. 63:344–352, 1972.

DISCUSSION

Chairman Frank J. Veith: Have you correlated the findings that you've presented with the simple parameters of white count and temperature? It would appear from our observations in man that temperature is an excellent index of early acute rejection with lung and kidney allografts. Is the same true of heart allografts, or is the EKG a beautiful and sensitive index clinically?

Dr. Stinson: Several years ago Dr. Lower and Dr. Dong of Dr. Shumway's laboratory proved the electrocardiogram to be the most reliable index of rejection in animals. The initial studies in the early 1960's on the correlation of white blood cell count, lymphocyte percentages, and dog temperatures were quite useless in monitoring rejection. I'm afraid that the picture is clouded in man, as it usually is, by the variety of clinical variables. For instance, we find that the administration of antilymphocyte or antithymocyte globulin is so regularly associated with fever that it completely abrogates body temperatures as any index of hematologic response.

CHAIRMAN VEITH: Have you done any studies comparable to the one you presented on immunosuppressed animals? It would appear that the process in humans, though similar, is just different enough to make a difference with the immunosuppressant drugs.

DR. STINSON: We're doing those studies now. They will be of great interest because there are, I think, noninvasive techniques for patient monitoring which can get at the systolic and diastolic wave forms of left ventricular function in order to correlate the changes seen in electrocardiographic voltage—a very sensitive index of function changes. We've just begun to evaluate resolution of these changes in systolic and diastolic performance with successful immunosuppressive therapy.

CLAUDE CHARTRAND, St. Gusting Hospital, Montreal: Did you differentiate electronically the aortic flow signal, and if so, did you get any informative data on acceleration?

DR. STINSON: No we didn't, but I expect that if we had, we would have found changes exactly as you found earlier. There is a suggestion from some experiments that the acceleration of aortic flow does approach a meaningful index of contractility, although it may not be perfect. In his earlier studies, Dr. Chartrand found that the differentiated aortic flow pattern was a more sensitive index of left ventricular performance than was peak velocity or total stroke output.

CHAPTER VIII

PHARMACOLOGY

The Pharmacokinetics of Amphetamine in Domestic Animals

The Use of the Animal Model in Assessing Analgesic Potency and Dependence Liability

Pharmacologic Actions of Newer Beta Blocking Agents

Comparative Pharmacokinetics in Domesticated Animals

The Actions of Cardioactive Drugs on Developing Myocardium

Drug Studies on Isolated Animal and Human Cardiac and Vascular Tissues: Advantages and Limitations

A Study of Cell Kinetics in the Rat Thymus After Perturbation By Dexamethasone

Application of Miniature Swine to Anesthetic Studies on the Cardiovascular System and Hepatic Inhalation Anesthetic Metabolism

THE PHARMACOKINETICS OF AMPHETAMINE IN DOMESTIC ANIMALS

J. D. Baggot and L. E. Davis*

The objective of this work was to determine the pharmacokinetic constants describing the distribution and elimination of amphetamine in different species of domestic animals. The species studied were ponies, goats, swine, dogs, cats, rabbits and chickens. A similar dose (0.66 mg/kg) of *dl*-amphetamine sulphate was administered i.v. to each experimental subject. Amphetamine concentrations in biological fluids (blood plasma, cerebrospinal fluid, urine and bile) were measured by a sensitive and specific gas chromatographic method. The drug attained distribution equilibrium very rapidly and elimination was first-order in all species. Plasma half-life values varied from 0.62 hour in goats to 6.53 hours in cats. The mean biological half-life of the drug varied significantly among species (F-test, $p<0.01$). Extent of plasma protein binding *(in vitro)* was determined by equilibrium dialysis technique. The percent amphetamine bound to plasma proteins was independent of drug concentration within the range of concentrations observed *in vivo* (25–400 ng ml^{-1}). Extent of binding was low (<41%) in all species but varied significantly among species (F-test, $p<0.01$). The apparent specific volumes of distribution of the drug, corrected for extent of plasma protein binding, were large (>2.1 litres/kg) in all species, this indicated extensive tissue distribution and sequestration. Approximately one-third of the amphetamine injected was excreted unchanged in urine of carnivorous animals and chickens while a small percentage of the dose was excreted unchanged in urine of herbivorous animals. The mean biological half-lives of the drug were significantly different in intact and nephrectomized dogs (Student's "t" test, $p<0.001$). Biliary excretion of unchanged drug was low. A relationship appeared to exist between dietary habit and elimination of amphetamine. Elimination was significantly slower in carnivorous species than in the herbivorous and omnivorous species studied.

INTRODUCTION

The amphetamine molecule has a simple chemical structure, diffuses rapidly into tissues of high blood perfusion, undergoes a complex metabolic fate, and markedly alters the physiologic disposition of norepinephrine. The drug is eliminated from the body by excretion and biotransformation. Amphetamine (2-amino-1-phenylpropane) can be metabolized along two major pathways, either by hydroxylation of the aromatic ring to p-hydroxyamphetamine or by deamination of the side chain to 1-phenylpropane-2-one (benzyl methyl ketone), which can then be degraded to benzoic acid. These two pathways are shown to occur and their relative extent appeared to vary with species.[1-8] A minor pathway for amphetamine metabolism involved β-hydroxylation;[9] this was catalyzed by the enzyme dopamine β-oxidase.[10] Little is known, however, about the species distribution of the β-hydroxylation reaction.[11]

The pKa value of *d*-amphetamine is 9.90;[12,13] this implies that at the physiological pH only a small fraction (0.31 per cent) is in the neutral or nonionized form. The neutral form is reasonably soluble in various organic solvents whereas the ionized form is water-soluble. The lipid-solubility of the neutral form is reflected in the so-called true partition coefficient, for example, hepatane/water 1:88; chloroform/water 1:46.[14]

The objective of this work was to determine the pharmacokinetic constants describing the distribution and elimination of amphetamine in different species of domestic animals. Prior to this study, curves of the decline of plasma amphetamine concentration were not available, so it was unknown whether elimination was a simple first-order or multiphasic process. Elimination refers to all the processes that operate to reduce the effective drug concentration in the body fluids.

* Division of Comparative Pharmacology, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210.

MATERIALS AND METHODS

Experimental Procedures

The experimental subjects comprised randomly selected groups of healthy animals of both sexes from each of six domesticated mammalian species and chickens. A similar dose (0.66 mg/kg, calculated as free base) of *dl*-amphetamine sulphate (Amfetasul, Pitman-Moore, Indianapolis, Ind.) was administered intravenously to each subject. Venous blood samples were collected in tubes, containing dipotassium etylenediamine tetraacetate, at fixed time intervals. Plasma was separated by centrifugation and stored at −10° pending analysis. The rate of elimination and extent of distribution of amphetamine were studied in Shetland-cross ponies[5], Toggenburg goats[10], Yorkshire swine[9], New Zealand White rabbits[4], mongrel dogs[11], American Short-haired cats[6] and Leghorn chickens[8]. Experiments were performed to determine quantitatively the unchanged amphetamine, p-hydroxyamphetamine and its conjugates in cumulative urine samples. A urine collection period which exceeded five half-lives of the drug in each species was employed. The volume and pH of the urine were measured.

The amounts of unchanged amphetamine excreted in urine and bile of dogs and swine were measured. The influence of nephrectomy upon the rate of elimination and extent of distribution of amphetamine was studied in nephrectomized dogs. Under pentobarbital anaesthesia eight randomly selected mongrel dogs were bilaterally nephrectomized. The BUN values (mean ± S.E.) in five dogs 24 hours after nephrectomy were 88 ± 6.1 mg/100 ml (normal, 10–30 mg/100 ml). Amphetamine was administered 42–48 hours after surgical removal of the kidneys.

The extent of plasma protein binding was determined *in vivo* by measuring the amphetamine concentrations in cerebrospinal fluid and plasma samples collected simultaneously after distribution was complete. The experimental subjects were seven healthy and eight nephrectomized dogs.

Blood, free of drug, was collected in tubes containing dipotassium EDTA from donor animals of the several species used in this study. Plasma was separated by centrifugation. The total plasma protein concentration was measured by refractometry, the plasma was then stored at −10° until used in determining the extent of amphetamine protein binding *(in vitro)*.

Analytical Techniques

The amphetamine concentration in the biological fluids was measured by a sensitive and specific gas chromatographic method.[15] Amphetamine was extracted from alkaline biologic fluid into cyclohexane. The trichloroacetamide derivative was prepared with trichloroacetyl chloride in the organic phase. This derivative was chromatographed on 3% OV-1 and detected by electron capture (Beckman Gas Chromatograph, Model GC-5). The concentration of amphetamine in biological fluids of medicated animals was estimated by comparing sample peak heights with standard peak heights on the chromatogram. The standard solutions were analyzed in the same manner as the samples to compensate for losses occurring in the procedure.

The concentration of p-hydroxyamphetamine in urine was determined by the colorimetric method of Axelrod.[2] The glucuronide and sulphate conjugates of p-hydroxyamphetamine were assayed as the latter following incubation of urine with appropriate enzyme in a suitable buffer. Glucuronide conjugate: to 3 ml 0.2 M acetate buffer (pH 4.6) was added 3 ml urine and 50 mg β-glucuronidase (beef liver, salt free, 70,000 to 100,000 units per 10 g; Nutritional Biochemicals Corporation, Cleveland, Ohio). Sulphate conjugate: to 3 ml 0.2 M acetate buffer (pH 5.0) was added 3 ml urine and 1 unit sulfatase (Sulfatase, Type III: from Limpets; Sigma Chemical Company, St. Louis, Mo.). The mixtures were incubated for one hour at 37° in a Dubnoff metabolic shaking incubator. Following this period the total p-hydroxyamphetamine concentration was determined in 2.5 ml of the incubated mixture. The concentration of conjugate was estimated by difference between total and unconjugated hydroxyamphetamine concentrations.

The equilibrium dialysis technique using ^{3}H-*d*-amphetamine sulphate (New England

Nuclear) and liquid scintillation counting (Packard Tri Carb Model 3380 liquid scintillation spectrometer) was employed to measure the extent of plasma protein binding *in vitro*. The extent of protein binding was determined at a drug concentration of 1×10^{-6} M (activity 0.156 μ Ci/ml).

RESULTS AND DISCUSSION

The disappearance of amphetamine from the blood plasma of the various species is shown (Figure 1). The distribution of this drug was rapid, less than thirty minutes, and elimination followed first-order kinetics in all species. Values for the kinetic constants are tabulated in Table I. The mean biological half-life under normal conditions of fluctuating urinary pH in these randomly selected groups of animals varied among species (F-test, $p<0.01$). The shortest half-life of this drug was in goats; the half-life was of intermediate duration in ponies, rabbits, chickens and swine, while dogs and cats had longer half-lives. No sex difference in persistence of the drug was observed in any species studied. The value B may be defined as the concentration of drug which would be found in the plasma at time zero if the distribution of drug to tissue fluids occurred instantaneously. On extrapolating to zero time (Figure 1), the mean values for B varied from 235 ng ml^{-1} in goats to 438 ng ml^{-1} in chickens.

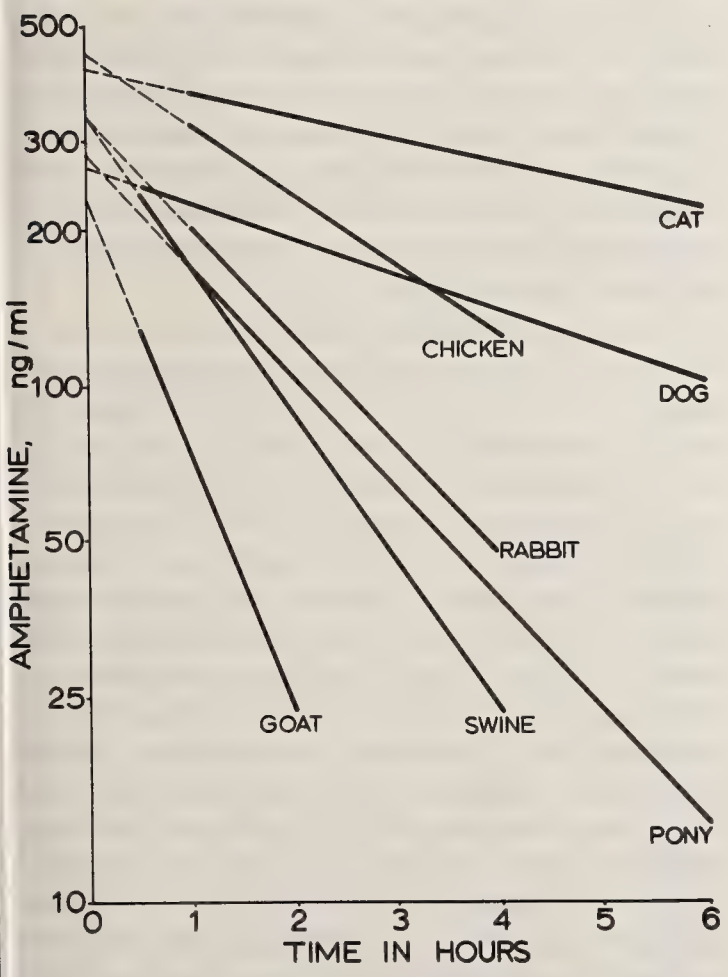


FIGURE 1.—Disappearance of amphetamine from the blood plasma of several species following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Elimination was first-order in all species.

The assay procedure measured both free and bound amphetamine in plasma samples and since only unbound drug was available for diffusion out of the circulation, the apparent volume of distribution had to be corrected for the extent of plasma protein binding. The percent amphetamine bound to plasma proteins was independent of drug concentration within the range of concentrations studied (2.5×10^{-7} M to 4.0×10^{-6} M). Extent of protein binding independent of drug concentration was reported for desmethylimipramine[16] and for amphetamine[17] in human plasma. Preliminary work suggests that the extents of plasma protein binding of chloramphenicol and morphine in an animal species are independent of drug concentration within the therapeutic range of plasma levels. In contrast, the extent of plasma protein binding of quinidine was concentration dependent.[18]

The extent of binding of amphetamine (mean ± S.E.) at a drug concentration of 1×10^{-6} M and the total plasma protein concentration for

TABLE I.—*Pharmacokinetic Constants (Mean ± S.E.) Describing the Distribution and Elimination of Amphetamine in Different Species of Domestic Animals*

Species (number)	B (ng/ml)	$t_{1/2}$ (hr)	$\beta-1$ (hr)	(litre/kg) V'd
Goat (10)	235 ± 15	0.62 ± 0.04	1.118 ± 0.08	3.08
Swine (9)	330 ± 15	1.05 ± 0.05	0.654 ± 0.03	2.23
Pony (5)	281 ± 34	1.39 ± 0.08	0.499 ± 0.03	2.61
Rabbit (4)	333 ± 8	1.40 ± 0.08	0.498 ± 0.03	2.29
Chicken (8)	438 ± 15	2.27 ± 0.17	0.305 ± 0.03	1.81
Dog (11)	263 ± 10	4.50 ± 0.24	0.159 ± 0.01	2.67
Cat (6)	401 ± 5	6.53 ± 0.39	0.108 ± 0.01	1.83

B = Theoretical drug concentration in plasma at time zero
$t_{1/2}$ = Biological half-life
β = Overall elimination rate constant
V'd = Apparent specific volume of distribution

TABLE II.—*Total Plasma Protein Concentration and Extent of Protein Binding of Amphetamine in Different Species of Animals*

Species	% bound amphetamine Mean ± S.E.	Plasma protein conc. (g./100 ml) Mean ± S.E.
Goat (12)	40.7 ± 1.7	6.6 ± 0.3
Swine (6)	39.6 ± 3.0	6.4 ± 0.6
Pony (9)	25.3 ± 2.6	7.5 ± 0.3
Rabbit (4)	31.0 ± 2.9	5.5 ± 0.3
Chicken (6)	14.5 ± 0.9	3.0 ± 0.3
Dog (17)	27.1 ± 1.5	6.7 ± 0.3
Cat (12)	26.4 ± 1.2	7.3 ± 0.2

Amphetamine concentration: 10^{-6}

each species are tabulated in Table II. Species differences in the extent of protein binding of the drug were observed (F-test, $p<0.01$). At a similar drug concentration the extent of binding (mean ± S.E.) in dependent and drug naive human subjects was 23% ± 1.1 and 26% ± 1.0, respectively.[17] The extent of binding in chickens was lower than in any mammalian species; this feature might be explained by the low concentration of plasma proteins in the bird.

The mean percent bound amphetamine for each species was used to correct the B values for the individual animals within the species. The corrected apparent specific volumes of distribution were then computed. Dominquez[19] defined volume of distribution as the volume of body fluids which holds the substance in solution at the same concentration as the plasma. The corrected apparent specific volumes of distribution (V'd*) were large in all species (Table III), the magnitude of this parameter was indicative of extensive tissue distribution

TABLE III.—*Extent of Plasma Protein Binding, Corrected Apparent Specific Volume of Distribution and Overall Clearance Value of Amphetamine in Different Species of Domestic Animals*

Species (number)	% bound Amphetamine	10^{-6} M V'd* (litre/kg)	$C'_{overall}$ (ml min^{-1} kg^{-1})
Goat (10)	40.7	5.19	96.7
Swine (9)	39.6	3.69	40.6
Pony (5)	25.3	3.43	29.1
Rabbit (4)	31.0	3.32	27.4
Chicken (8)	14.5	2.12	14.6
Dog (11)	27.1	3.66	9.2
Cat (6)	26.4	2.49	4.4

V'd* = Apparent specific volume of distribution corrected for extent plasma protein binding of drug

$C'_{overall}$ (i.e., overall clearance value per kilogram) = β x V'd*

and sequestration of the drug. Axelrod[2] examined the distribution of *d*-amphetamine in representative tissues of a dog which was given 10 mg/kg of the drug intraperitoneally. The animal was sacrificed one hour after the drug was administered. The drug was found to be distributed in most organ tissues but only negligible amounts were present in fat and bile. Similar tissue distributions of this drug were reported in the mouse,[20] rat,[21] and cat.[22] Most of the drug was shunted toward tissues of high blood perfusion, that is, kidneys, lungs, brain and liver. The very large apparent specific volume of distribution in goats (5.19 litres/kg) was believed to be due to rapid diffusion and subsequent trapping of amphetamine in the rumen. The concentration of amphetamine was higher (ca. 50%) in rumen fluid than in plasma thirty minutes after i.v. administration of drug. The pH gradient across the reticuloruminal epithelium (ca. 1 pH unit) would favor a passive transfer process, for example, nonionic diffusion. Ephedrine attained concentrations in rumen liquor which were several times greater than the corresponding plasma levels following intravenous infusion of this compound.[23] Quinine also diffused from plasma into ruminal fluid and was trapped there.[24]

The overall clearance value is an estimate of the sum of the metabolic and excretory clearances of drug from plasma water. Values for overall clearance varied from 4.4 ml min^{-1} kg^{-1} in cats to 96.7 ml min^{-1} kg^{-1} in goats. The overall clearance values in the other species studied were intermediate between these extremes. The relative amounts of amphetamine excreted in urine and bile of dogs, swine and rats[25] indicated that urinary excretion of unchanged drug was important but biliary excretion was low. The dose of amphetamine administered, the biological half-life of the drug and the cumulative amounts of unchanged amphetamine excreted in urine and bile of individual swine are tabulated in Table IV. The mean cumulative amounts of unchanged amphetamine excreted in urine and bile were 8.18 and 0.12 percent of the dose, respectively. The appearances of the drug in urine and bile followed first-order kinetics (Figure 2). The dose of amphetamine administered, the urinary pH, the

TABLE IV.—*The Dose of Amphetamine Injected I.V., the Biological Half-life of the Drug and the Cumulative Amounts of Amphetamine Excreted in Urine and Bile of Swine*

Subject pig	Total dose (mg)	Urine pH mean value (range)	$t_{1/2}$ (hr)	Cumulative amount of amphetamine (3 hr) Urine (mg)	Bile (μg)
PB	13.2	5.87 (5.71–6.05)	0.93	1.08	14.4
PC	16.2	5.82 (5.61–5.76)	1.14	1.36	20.3
PX	11.8	6.07 (5.88–6.34)	0.92	0.93	14.9

biological half-life of the drug, and the cumulative amounts of unchanged amphetamine excreted in urine and bile of individual dogs are tabulated in Table V. No attempt was made to control urinary pH; the observed values were within the normal range for the canine species. Despite individual differences in biological half-life there appears to be a direct relationship between urinary pH and the biological half-life of this drug; the more acid the urine, the shorter the biological half-life. The renal clearance values in dogs (2.8 to 5.1 ml min^{-1} kg^{-1}) provided evidence that amphetamine probably undergoes glomerular filtration and tubular reabsorption. The amount of unchanged

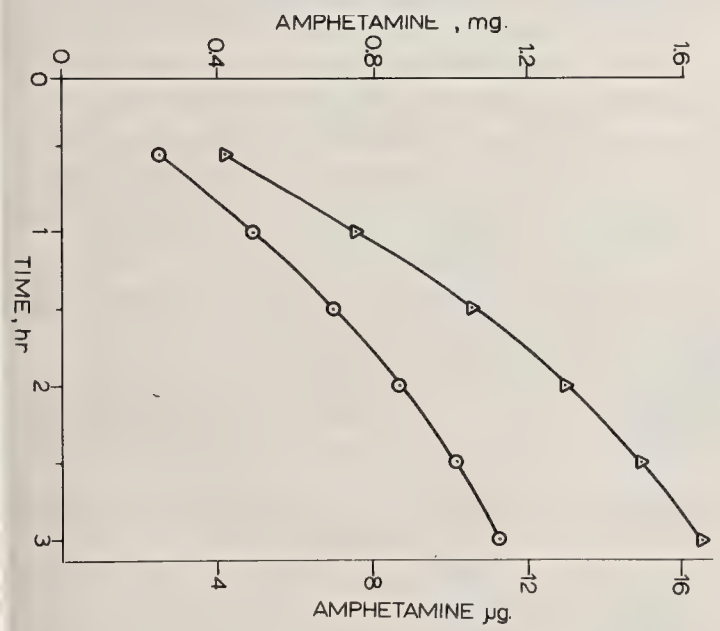


FIGURE 2.—The cumulative appearance of amphetamine in urine ○——○ (mg) and bile △——△ (μg) of swine following the intravenous injection of amphetamine sulphate (0.66 mg/kg, free base). Each point represents the mean amount of amphetamine in the biological fluid of three animals.

amphetamine in urine was approximately five hundred times the amount present in bile in each experimental subject. A substantial portion of the dose was excreted in the urine of man unchanged,[26,27] and urinary pH was an important factor in determining the rate of excretion of this drug.[28,29] In man, amphetamine was cleared from blood more rapidly than could be accounted for by glomerular filtration under acid conditions, but when urinary pH fluctuated, clearance of the drug could be accounted for by this route.[30] Unlike man and the dog, the biological half-life of this drug in the horse was independent of urinary pH. This was probably due to the small amount of nonionized amphetamine available for renal tubular reabsorption.

The quantities of unchanged amphetamine, p-hydroxyamphetamine and its glucuronide and sulphate conjugates in cumulative urine samples of the several species are summarized in Table VI. Comparison of urinary pH of the species suggests that differences in the amount of amphetamine excreted unchanged cannot be related to variations in urinary pH. Although this may be a contributing factor, the rate of the principal metabolic pathway for this drug is most likely to be the dominant factor determining the proportion of the dose excreted unchanged. The amounts of the various fractions recovered, expressed as percent of the dose administered, are shown in the histogram form (Figure 3). Approximately one-third of the amphetamine injected was excreted unchanged in urine of carnivorous animals (dog and cat) and the chicken while a relatively small fraction of the dose was excreted unchanged in urine of herbivorous species (pony, rabbit and goat). The low percent of dose recovered in urine of ponies (33%) and rabbits (27%) suggested that the principal metabolite was not measured. Oxidative deamination was shown to be the major metabolic pathway for amphetamine in the rabbit.[1,3,6-8] Swine excreted 15% of the dose unchanged and the rat 13% of the dose in 48 hours.[8] The rates of disappearance of amphetamine from plasma of swine and male rat[31] were similar. Also the principal metabolic pathway in these two omnivorous species was p-hydroxylation and glucuronidation.

TABLE V.—*The Dose of Amphetamine Injected I.V., the Biological Half-life of the Drug and the Cumulative Amounts of Amphetamine Excreted in Urine and Bile of Dogs*

Subject dog	Total dose (mg)	Urine pH mean value (range)	$t_{1/2}$ (hr)	Cumulative amount of amphetamine (4 hr) Urine (mg)	Bile (μg)
R	13	7.50 (7.38–7.76)	6.13	1.25	3.79
T	10	5.96 (5.92–6.00)	3.67	2.10	5.00*
V	10	6.55 (6.48–6.61)	4.25	1.77	5.50
X	10	6.51 (6.48–6.54)	4.18	1.80	6.87
Z	13	6.28 (6.20–6.38)	3.85	3.25	6.50

* = 3 hours

The plasma amphetamine concentration *versus* time profiles in intact and nephrectomized dogs are shown in Figure 4. The biological half-life was extended by almost 25% following nephrectomy and the difference between the mean half-lives in intact and nephrectomized dogs was statistically very highly significant (Student's "t"-test, $p<0.001$). Nephrectomy did not significantly change the corrected apparent specific volume of distribution of this drug. The extent of protein binding (mean ± S.E.) in tact (23.1% ± 1.7) and nephrectomized (24.3% ± 1.5) dogs was not significantly different (Student's "t"-test, $p>0.05$). It thus appears that uremia had no effect upon the extent of plasma protein binding of amphetamine. Desmethylimipramine had almost normal binding in plasmas from uremic patients while the extent of binding of diphenylhydantoin was reduced.[32] The extents of protein binding in the dog determined by the *in vitro* equilibrium dialysis technique and by measuring *in vivo* the amphetamine concentrations in plasma and cerebrospinal fluid collected simultaneously were not significantly different (Student's "t"- test, $p>0.05$, Table VII).

SUMMARY

A relationship appeared to exist between dietary habit of the various species of animals and the following observations.

1. *Biological Half-life of the Drug.* The half-life was significantly longer in carnivorous species (dog and cat) than in herbivorous (pony, rabbit and goat) and omnivorous (swine, rat[31] and chicken) species, except man.[27]
2. *Metabolic Pattern.*[11] Herbivorous species metabolized the phenylisopropylamine group largely by oxidative deamination. Carnivorous and omnivorous species utilized aromatic hydroxylation as well as oxidative deamination.

Some similarities were observed in the fate of amphetamine among the several species of animals. The rapid attainment of distribution equilibrium, low extent of plasma protein binding and large apparent volume of distribution were characteristics of this drug in all species. While the proportion of dose excreted unchanged in urine varied considerably among species, biliary

TABLE VI.—*Percent Dose (Mean ± S.E.) of Amphetamine and of Its Metabolites in 24-Hour Urine of Different Species*

Species (number)	Urinary pH reaction (range)	Amphetamine	4-Hydroxy-amphetamine	Conjugates		Percent dose recovered
Goat (10)	7.50 (6.07–8.40)	12 (1.5)	11 (1.0)	13 (2.3)	21 (2.0)	57
Swine (6)	6.85 (6.26–8.50)	15 (1.5)	20 (2.5)	27 (3.1)	3 (0.4)	65
Pony (4)	8.06 (7.70–8.32)	2	12	13	6	33
Rabbit (9)	8.00 (7.56–8.49)	3 (0.4)	10 (0.9)	8 (0.08)	6 (0.4)	27
Chicken (4)	6.56 (6.23–6.92)	32 (3.8)	7 (1.0)	7 (2.2)	12 (1.0)	58
Dog (6)	7.45 (6.65–7.75)	30 (1.2)	11 (2.2)	4 (0.6)	3 (0.4)	48
Cat (8)*	7.35 (6.45–8.00)	35 (2.2)	5 (0.3)	9 (0.8)	10 (0.4)	59

* 48-hour urine

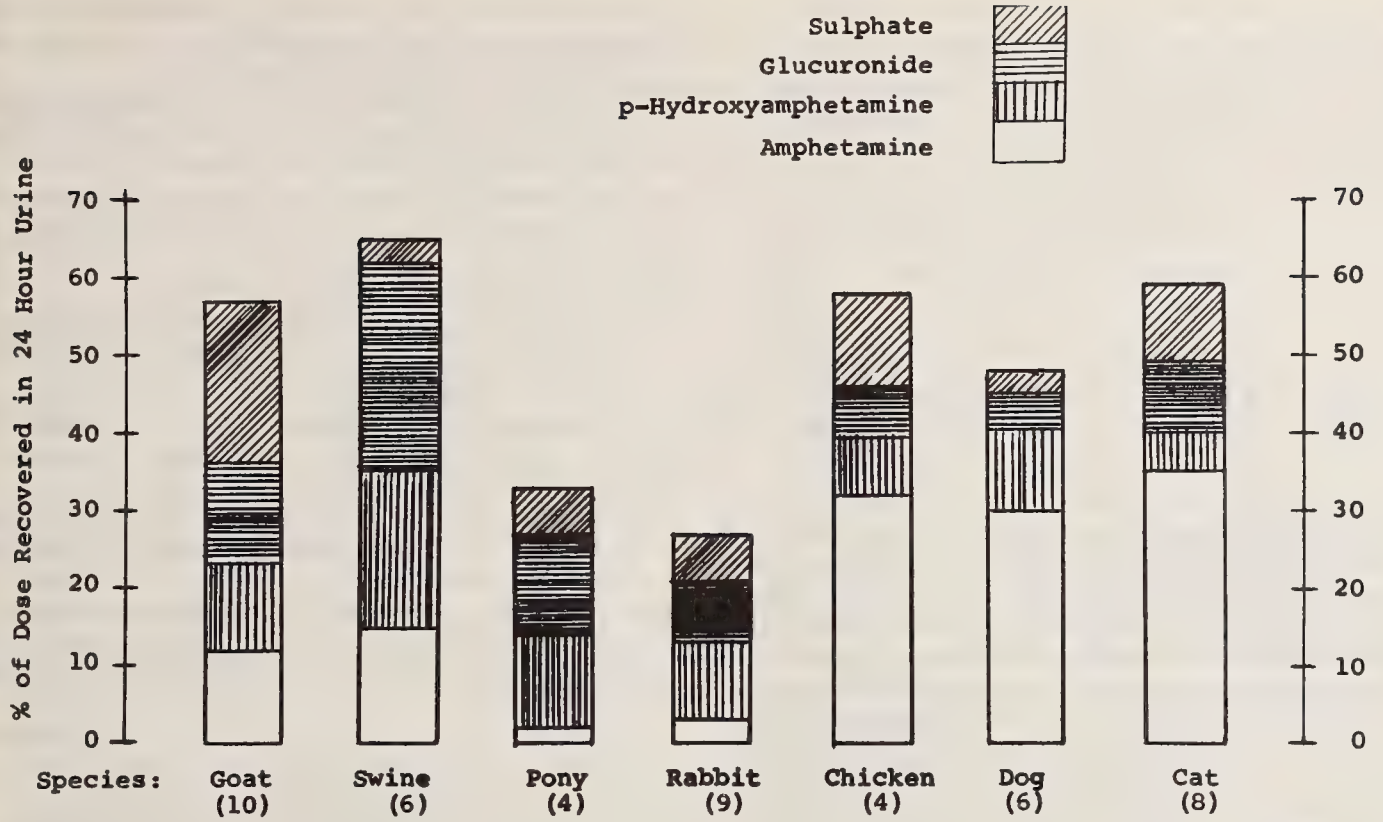


FIGURE 3.—The percent of dose of amphetamine, p-hydroxyamphetamine and its conjugates in cumulative urine (24 hours in all species except cats, 48 hours) of different species. The number in parentheses is the number of animals studied in the species.

excretion of unchanged amphetamine was low in all species examined (dog, swine, rat,[25] and man[26]).

REFERENCES

1. AXELROD, J. An enzyme for the deamination of sympathomimetic amines. Properties and distribution. J. Pharmac. Exp. Ther. 110:2, 1954.
2. AXELROD, J. Studies on sympathomimetic amines. II The biotransformation and physiological disposition of *d*-amphetamine, *d*-p-hydroxyamphetamine and *d*-methamphetamine. J. Pharmac. Exp. Ther. 110:315–326, 1954.

TABLE VII.—*Plasma Protein Binding of Amphetamine in Dogs*

Variable	% Bound Amphetamine	
	in vitro	*in vivo**
Amphetamine conc.	135 ng ml^{-1}	100–150 ng ml^{-1}
Mean	27.1	23.7
n	17	15
S.E.	1.5	1.1

Student's "t" test: n.s. ($p > 0.05$)

* Experimental subjects were 7 healthy and 8 nephrectomized uremic dogs.

3. AXELROD, J. The enzymatic deamination of amphetamine (Benzedrine). J. Biol. Chem. 214:753–763, 1955.
4. ALLEVA, J. J. Metabolism of tranylcypromine-^{14}C and *dl*-amphetamine-^{14}C in the rat. J. Med. Chem. 6:621–624, 1963.
5. ELLISON, T., GUTZAIT, L., and VAN LOON, E. J. The comparative metabolism of *d*-amphetamine-^{14}C in the rat, dog and monkey. J. Pharmac. Exp. Ther. 152:383–387.
6. DRING, L. G., SMITH, R. L., and WILLIAMS, R. T. The fate of amphetamine in man and other mammals. J. Pharm. Pharmac. 18:402–404, 1966.
7. DRING, L. G., SMITH, R. L., and WILLIAMS, R. T. A precursor of benzyl methyl ketone in amphetamine urine. Biochem. J. 109:10P–11P, 1968.
8. DRING, L. G., SMITH, R. L., and WILLIAMS, R. T. The metabolic fate of amphetamine in man and other species. Biochem. J. 116:425–435, 1970.
9. GOLDSTEIN, M., and ANAGNOSTE, B. The conversion *in vivo of d*-amphetamine to (+)-p-hydroxynorephedrine. Biochim. Biophys. Acta. 107:166–168, 1965.
10. GOLDSTEIN, M., and CONTRERA, J. F. The substrate specificity of phenylamine-β-hydroxylase. J. Biol. Chem. 237:1898–1902, 1962.
11. SMITH, R. L., and DRING, L. G. Patterns of metabolism of β-phenyl-isopropylamines in man and

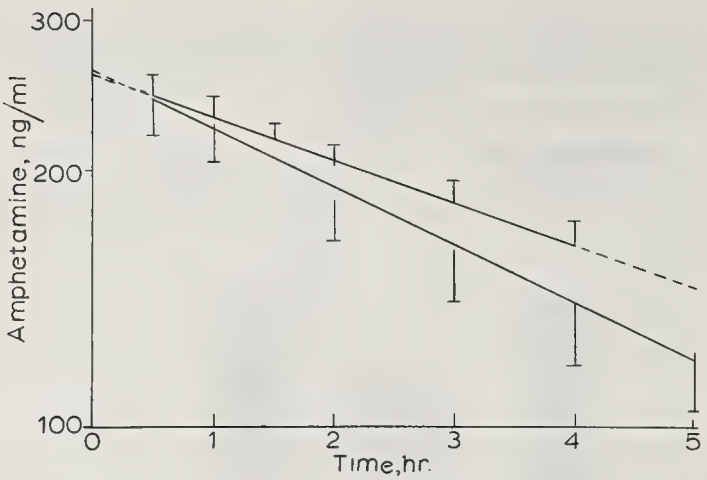


FIGURE 4.—Disappearance of amphetamine from the blood plasma of intact (lower line) and nephrectomized (upper line) dogs following the intravenous injection of amphetamine sulphate (0.66 mg/kg, free base). Each point represents the mean plasma amphetamine concentration and each vertical bar is 1 S.D. The mean values of the pharmacokinetic parameters were calculated: intact dogs (n = 11), B = 263 ng ml^{-1}, t½ = 4.50 hours, V'd* = 3.58 litres kg^{-1}, $C'_{overall}$ = 9.18 ml min^{-1} kg^{-1}; nephrectomized dogs (n = 8), B = 258 ng ml^{-1}, t½ = 5.69 hours, V'd* = 3.38 litres kg^{-1}, $C'_{orveall}$ = 6.88 ml min^{-1} kg^{-1}.

other species. In: *International symposium on amphetamines and related compounds.* Edited by E. Costa and S. Garattini. New York: Raven Press, pp. 121–138, 1970.

12. LEFFLER, E. B., SPENCER, H. M., and BURGER, A. Dissociation constants of adrenergic amines. J. Am. Chem. Soc. 73:2611–2613, 1951.
13. LEWIS, G. P. The importance of ionization in the activity of sympathomimetic amines. Br. J. Pharmac. 9:488–493, 1954.
14. VREE, T. B., MUSKENS, A. T., and VAN ROSSUM, J. M. Some physico-chemical properties of amphetamine and related drugs. J. Pharm. Pharmac. 21:774–775, 1969.
15. NOONAN, J. S., MURDICK, P. W., and RAY, R. S. A method for detecting amphetamine using gas chromatography of a halogenated derivative. J. Pharmac. Exp. Ther. 168:205–209, 1969.
16. BORGA, O., AZARNOFF, D. L., PLYM FORSHELL, G., and SJOGVIST, F. Plasma protein binding of tricyclic antidepressants in man. Biochem. Pharmac. 18:2135–2143, 1969.
17. FRANKSSON, G., and ANGGARD, E. The plasma protein binding of amphetamine, catecholamines and related compounds. Acta Pharmac Tox. (Kbh.) 28:209–214, 1970.
18. NEFF, C. A., DAVIS, L. E., and BAGGOT, J. D. A comparative study of the pharmacokinetics of quinidine. Am. J. Vet. Res. (In press) 1971.
19. DOMINQUEZ, R. Kinetics of elimination, absorption and volume of distribution in the organism. Med. Physics II 476–489, 1950.
20. FULLER, R. W., and HINES, C. W. Tissue levels of chloroamphetamines in rats and mice. J. Pharm. Sci. 56:302–303, 1967.
21. BRZEZINSKI, J. Rate of amphetamine absorption and excretion in rats. Acta Pol. Pharm. 25:91–96, 1968.
22. SIEGEL, M., ELLISON, T., SILVERMAN, A. G., and OKUN, R. Tissue distribution of *dl*-^{3}H-amphetamine HCl in tolerant and nontolerant cats. Proc. West. Pharmac. Soc. 11:90–94, 1968.
23. CORKER, E. *The secretion of certain drugs into the bovine rumen-reticulum.* M.S. Thesis, University of Minnesota, Minneapolis, 1966.
24. JENKINS, W. L. *Drug distribution across ruminal epithelium.* Ph.D. Thesis, University of Missouri, Columbia, 1969.
25. CALDWELL, J., DRING, L. G., and WILLIAMS, R. T. The biliary excretion of amphetamine and methamphetamine in the rat. Biochem. J. 124:16P, 1971.
26. BECKETT, A. H., and ROWLAND, M. Urinary excretion kinetics of amphetamine in man. J. Pharm. Pharmac. 17:628–639, 1965.
27. ROWLAND, M. Amphetamine blood and urine levels in man. J. Pharm. Sci. 58:508–509, 1969.
28. ASATOOR, A. M., GALMAN, B. R., JOHNSON, J. R., and MILNE, M.D. The excretion of dexamphetamine and its derivatives. Br. J. Pharmac. 24:293–300, 1965.
29. BECKETT, A. H., ROWLAND, M., and TURNER, P. Influence of urinary pH on excretion of amphetamine. Lancet. i:303, 1965.
30. BECKETT, A. H., SALMON, J. A., and MITCHARD, M. The relation between blood levels and urinary excretion of amphetamine under controlled acidic and under fluctuating urinary pH values using ^{14}C-amphetamine. J. Pharm. Pharmac. 21:251–258, 1969.
31. GROPPETTI, A., and COSTA, E. Factors affecting the rate of disappearance of amphetamine in rats. Int. J. Neuropharmac. 8:209–215, 1969.
32. REIDENBERG, M. M., ODAR-CEDERLOF, I., VON BAHR, C., BORGA, O., and SJOQVIST, F. Protein binding of diphenylhydantoin and desmethylimipramine in plasma from patients with poor renal function. N. England J. Med. 285:264–267, 1971.

DISCUSSION

CHAIRMAN COHEN: Dr. Baggot's interesting paper is now open for discussion.

JULES CASS, Veteran's Administration, Washington, D.C.: These are one dose experiences, are they? One wonders, of course, in the repeated dose, what sorts of shifts occur in the

various species? This of course is a very apparent thing. My other question concerns the experience with these drugs diurnally and nocturnally because some of these animals are not daytime animals; some of them are nighttime animals, or not specifically daytime. If we're talking as pharmacologists, the paper addresses itself to the diurnal. But I thought that it might be interesting to look at these experiments from the standpoint of the animal subject used. Of course in your conclusions you alluded to that. When one reads journals in pharmacology, a dog is a dog, a mouse is a mouse, and a rat is a rat. And those of us who have worked with these species recognize that the strains *within* the particular breeding colony have quite an impact on these kinds of studies and the results you obtain. I only speak about these things because I feel that this is the purpose of our meeting here.

CHAIRMAN: I appreciate your comments, Dr. Cass. Would you like to amplify those statements or in any way address yourself to them?

DR. BAGGOT: I'd just like to say that we did use a particular type of breed of animal within each species. We always use one breed within each species except for dogs. In dogs, we just used mongrels, randomly selected mongrel dogs. I do appreciate that there might be quite a difference between breeds, particularly with the greyhound and the beagle. We just use these mongrel dogs.

QUESTIONER: Dr. Baggot, I'd like to just ask a question about the data that you have on carnivorous animals. Do you think that, if you were to extrapolate data pertaining to urine pH and rate of drug excretion in these animals, this might have any relevance to man in terms of amphetamine over-dosage? Are you able to make any statement about that?

DR. BAGGOT: Yes, I think so. Of course in man it has been shown, principally by Beckett and Rowland, that about 30 to 40% of the amphetamine is excreted unchanged in man. This was similar to what we obtained in the carnivorous animals. The urinary pH has a marked influence on the half-life in the human which has been shown by others to be about twelve hours. We, too, showed urine pH to have an influence upon amphetamine half-life in the dog; such a high proportion of the dose was excreted unchanged. On the other hand, we showed in herbivorous animals that only about 2 to 3% of the dose was excreted unchanged. This implies that a change of urinary pH is certainly of therapeutic value in the correction of overdosage. This is very definite in man, of course, and in the carnivorous animals. I don't think it would have any effect in the herbivorous species.

QUESTIONER: Did you go on to acidify or alkalinize the urine in man?

DR. BAGGOT: Yes, to shorten the biological half-life you would acidify the urine and to bring it down to 6.0, or somewhere below 6.0.

STUART FRAZIER, Food and Drug Administration, Washington, D.C.: How would intravenous amphetamine half-life compare with oral administration? You used oral administration here, right?

DR. BAGGOT: No, all were administered intravenously.

DR. FRAZIER: Well, how would this compare with the IM injection for distribution and half-life and so forth?

DR. BAGGOT: I never administered it other than intravenously, but I know that Rowland showed that, given orally in the human, the absorption is very rapid. The absorption and distribution are both very rapid, being complete with, I think, fifteen or twenty minutes. The half-life should be unchanged.

THE USE OF THE ANIMAL MODEL IN ASSESSING ANALGESIC POTENCY AND DEPENDENCE LIABILITY

J. Cochin*

The pharmacological properties of the narcotic analgesics make it imperative to use animal screening techniques in order to assess their therapeutic usefulness and dangers. Animal models have been used for the assessment of the analgesic potency of the narcotic analgesics for many years, but their use for the assessment of dependence liability is relatively recent. Analgesic testing methods are usually carried out in mice or in rats although some have been developed for larger animals such as the guinea pig and the dog. Dependence-liability, on the other hand is usually evaluated in the monkey, although more recently techniques have been worked out to assess physical and perhaps psychological dependence in mice and rats. Among the procedures used in the evaluation of analgesic potency are those that utilize thermal, mechanical, chemical, electrical and behavioral techniques. They vary from a simple reflex response to a nociceptive stimulus to a complex behavioral response in a complicated operant setting. These procedures employ apparatus that may be a little more than a heated wire applied to the tail or may be the complex electronic circuitry necessary for sophisticated operant behavioral techniques. The assessment of dependence liability employs methods that use refined observational techniques as well as procedures utilizing self administration of drugs. The latter may be used to assess both physical and psychological dependence. The measurement of the withdrawal syndrome in animals serves as an excellent model for the signs of abstinence in man, and the correlation of results in animals and man is quite good. Although most of the screening for such properties is done in the monkey, both the rat and the mouse can serve as models that have some validity. The induced abstinence syndrome in animals is one of a few disease entities in animals that parallels very closely that seen in man.

INTRODUCTION

The use of animals as models for human disease states is a field that is fraught with difficulty both in execution and interpretation. This is especially true when one is concerned with central nervous system phenomena, where subjective feelings, set, and observer interpretation of supposedly objective data play an important role. Thus, while the measurement of respiratory depression or cardiac output might sometimes pose enormous technical difficulties, and the interpretation of the meaning of the results may be matters of discussion and controversy, a reduction of tidal volume is a reduction of tidal volume and an increase in blood pressure is an increase in blood pressure. On the other hand, in the field of psychoactive drugs we are faced not only with matters of technical difficulty, but, in the measurement of modalities that are partially if not wholly subjective, we are also faced with the question of the interpretation of whatever it is we are measuring by the experimental subject or patient and the reinterpretation of the observation by the observer or clinician.

This introduction of subjective coloring of the measured modality has led to much chagrin, frustration and rationalization on the part of those who work with psychoactive drugs. For instance, the experimental production of pain has posed immense philosophical as well as practical problems. Do animals feel pain as we humans understand it? Are we justified in equating a simple reflex to a noxious stimulus with pain as we understand it? Obviously we cannot ask the animal "does it hurt?". On the other hand, the experimental production of pain in humans also poses some rather intriguing problems, problems that have to do with suggestibility, with the fact that the subject knows that he can terminate the pain at will and with the set, the environment and the motivation. If pain is a difficult assessment to make in animals, consider the pitfalls encountered in the

* Boston University School of Medicine, Boston, Massachusetts.

assessment of drug-seeking behavior, of psychological dependence to drugs. Nevertheless, workers in the area of drug addiction believe that these assessments, although quite difficult, are not impossible, and a number of animal techniques that are both sensitive and reproducible with which to make these assessments have been developed.

Any discussion of screening methods for most classes of drugs is usually confined to assays which define and delimit their potency, their site of action, their mode of action if at all possible, their efficacy, their toxicity and so forth. The narcotic analgesics, however, must be assayed not only for the properties listed, but also for the properties that are not solely pharmacological but which also have major legal and sociological import and impact. The profile of activity of the opiate analgesics includes the ability to initiate in animals and in man physiological and psychological states which are referred to as physical and psychological dependence, respectively.

It is obvious that some of the effects produced by substances with addiction liability are demonstrable only through prolonged clinical and social experience. However, tolerance, physical dependence and to some degree psychological dependence can be measured quantitatively in man and fortunately in animals as well. It is to animal models of these states that this report will be directed.

There is little interest in the dependence liability of a new drug unless the compound in question is also a useful analgesic. Once this has been established, its abuse potential is of somewhat more than academic interest. Screening for analgesic potency is the first step in the long and complicated process of developing a new analgesic. A large number of techniques have been developed that are useful in assessing the antinociceptive activity, the activity that we refer to as analgesic activity in man. I shall outline briefly the principal methods used in the assay of analgesics in animals, those methods that utilize so-called nociceptive (noxious) stimuli to elicit a response in animals that we equate with a response to pain in man.

METHODS FOR ASSESSING ANALGESIA

The literature is filled with methods and polemic concerning these methods. All this seems to be beside the point since it becomes fairly obvious upon an examination of the literature that (a) there is no method that yields complete parallelism between results in animals and clinical practice in man, and (b) for each investigator one or another of several assay methods of antinociceptive activity is successful and yields results that are grossly comparable. As long as the results of a given assay technique are reproducible in one laboratory and are useful for that laboratory, then that method is a valid one. Whether or not we are actually measuring "pain threshold" or "analgesia", is not of great practical importance if we can relate the results to man and to the relief of his pain. A number of comprehensive reviews of this subject have appeared[1-3] and the reader is referred to them.

The animal techniques commonly used can be divided into thermal, mechanical, chemical and electrical and behavioral. The thermal methods are still the most widely used techniques in both the commercial and academic laboratory. All of them utilize the general principle of applying heat to the animal or human to elicit a response. The difference in time needed to elicit a response (a skin twitch, a tail flick, a lifting of a hind paw, a licking of a front paw) before and after administration of a drug is used as the measure of drug effect. This general method was adapted to the rat by D'Amour and Smith.[4] Heat is applied to the tail of a rat and the time that elapses before the rat flicks his tail away from the heat source is measured. Neither the original tail-flick method nor any of its many modifications have been able to assay with any degree of predictability in man the potency of compounds that have both agonist and antagonist activity, compounds such as nalorphine or levallorphan.

The tail-flick procedure has been modified to make it applicable to mice. In this modification, the tails are dipped into hot water and the end point is a flick or a rapid continuous movement of the tail.[5] Methods such as those described by Andrews and Workman[6] and by Winder[7] utilize the "skin-twitch" threshold after focusing

of radiant heat on the skin of the dog. These methods are the direct descendents of the Hardy-Wolff-Goodell technique[8] in man—methods that did not prove terribly successful in man and that are not much better in animals.

The last thermal technique that I will mention is that developed in its final form at NIH by Eddy and co-workers.[9] This is the hot-plate procedure. In this assay, rats or mice are placed on a plate that is heated to a constant temperature—55°C, for instance. The time to end point is either a lifting of the hind leg, or a licking of the front paws depending on the laboratory using this assay. In the hands of a competent, trained observer the results obtained by this method can be reproduced within a number of laboratories and are comparable between laboratories. It also has the advantage of a relatively long cut-off time since there is minimal tissue injury and the same animal can be tested a number of times—a procedure that is necessary in long-term studies of tolerance and dependence. Time-effect curves can be calculated over a period of many hours after injection of an analgesic, and changes in drug sensitivity can be followed with repeated testing for weeks or months.

In the course of studies of long-term effects of analgesic drugs and of the mechanisms involved in tolerance[10,11] thousands of hot-plate assays have been carried out by the author, his technicians, and his graduate students. The agreement of values obtained for areas under the time-effect curves between observers and over a period of years has been extremely good and has served to substantiate our opinion of the reliability and reproducibility of the assay in both the rat and the mouse. Since we are continually doing long-term studies of tolerance, it is important that the assay method itself should not add a variable in the form of tissue injury which would confound the results and confuse the experimenter. The hot-plate technique is one which results in minimal tissue damage even when used repeatedly on the same animals and which makes it possible to use a sufficiently long cutoff time so that small changes in drug effect can be measured. Its drawbacks, shared by the other thermal techniques, are that it is not sensitive to the effect of analgesics of the agonist-antagonist type nor will it give positive results with the non-narcotic analgesics of the salicylate class.

Heat is not the only noxious stimulus that can be used to elicit a pain-like response from animals. Pressure on the skin or distension of hollow viscera have been used for years. The tail pinch described by Haffner[12] and by Eddy[13] has the advantage of extreme simplicity of both apparatus and observation, but this is offset by the disadvantages of tissue injury and lack of precision of end point and stimulus, making repeated observations difficult. The end point is vocalization and there is some argument as to whether this is a better end point than the reflex movements elicited by other techniques.

One of the most ingenious variations on the mechanical technique was devised by Randall and Selitto.[14] These investigators increased the sensitivity of the rat to the pressure stimulus by injecting a yeast suspension into the hind paw and applying pressure to the inflamed area produced by the yeast injection. Both the narcotic analgesics and the salicylate-type analgesics increase the struggle threshold significantly —a struggle reaction being used as the end point rather than vocalization. The interpretation of the results is confounded by the fact that most of the non-narcotic analgesics have antiinflammatory properties as well, and there is no easy way of determining whether the drug acted by virtue of its antiinflammatory properties or its analgesic properties. Nevertheless, this technique is capable of detecting compounds of the salicylate type and the mechanism by which this is done is of theoretical rather than practical interest.

The chemical methods might be considered as logical outcomes of the yeast injection mentioned above. These assay procedures measure the writhing induced by the intraperitoneal injection of chemicals such as 2-phenyl-1,4-benzoquinone,[15] acetic acid,[16] bradykinin,[17] and hydrochloric acid[18] and the inhibition of this writhing by various analgesics. The "writhing test", which is also euphemistically called the "mouse peritoneal test", has caused a great deal of furor since writhing seems to offend the sensibilities of many investigators. Despite this it is

a widely used technique that has as its major advantage its sensitivity to the analgesic action of the narcotic antagonists and as its major drawbacks its lack of specificity, since many nonanalgesic drugs also inhibit writhing. Also there appears to be a lack of correlation with potency of analgesics in man. It is of interest to point out that the analgesic activity of the narcotic antagonists is one property of this class of compounds that is relatively easy to demonstrate in man[18] and extremely difficult to show in almost any animal model that can be devised.

Electrical methods, such as shock to the tooth pulp, or to the scrotal sac, or to the tail all suffer from serious drawbacks that make their use difficult. They give inconsistent results, low sensitivity and variable response with age, strain, sex, etc. However, if one combines nociceptive stimuli with a response that is more complex than vocalization, such as turning the head or lifting of the stimulated limb, one gets what are known as "behavioral" methods, and these range from very simple "flinch-jump" techniques[19] to very complex operant procedures. Many of the operant procedures involve producing conditioned anxiety[20]—a concept that must raise some eyebrows of investigators who find it difficult to conceive of anxious rats. Most of these procedures seem to tap properties of opiates other than analgesia and also give positive results with other CNS depressants.

There is, however, a modification of an operant procedure that may be suitable for the measurement of analgesic properties. This procedure, first described by Weiss and Laties,[21-23] is called either "fractional escape and avoidance" or "shock-threshold titration". In essence, this allows an animal to reduce the level of electric shock to a threshold which can be tolerated by pressing a bar, or in a later refinement, by turning a wheel.[24] The training time is minimal and even mice can be taught to lower the shock threshold in one training session of 15–30 minutes. The procedure can be adapted for use in the monkey[25] and the shock can be delivered not only through the grid floor on which the animal stands but to electrodes implanted in various discrete areas in the brain of the monkey and the rat. These techniques allow the expansion of the animal model and open the way for the study of reward and punishment areas in the cortex and midbrain and will allow us to make some inferences not only with regard to the site of action of the narcotic analgesics but with regard to the control of human behavior as well.

EVALUATION OF PHYSICAL DEPENDENCE

The assessment of addiction liability poses problems of a different and much more complicated nature. The nature of the addictive illness is such that it poses extremely difficult and perplexing problems for the clinician who has to treat it. Fortunately the disease model in animals is strikingly similar to that seen in man and much of our knowledge and thinking about tolerance, physical dependence and, more recently, psychological dependence and drug-seeking behavior have come from the animal model.

The evaluation of physical-dependence liability in animals uses procedures that were developed expressly by pharmacologists when they began to study the abstinence phenomena in depth. Many of the procedures in general use in animal studies closely resemble and may indeed be patterned after methods developed for use in the experimental investigation of physical dependence in man, while some of the experimental techniques in man were first developed in animals. The final screening of physical dependence liability is done in the monkey, but preliminary information can be obtained in smaller animals that has general applicability to man.

The establishment of the addiction research center at the University of Michigan has made possible the measurement of opioid-like properties in the monkey in a systematic and comprehensive manner and the results have consistently paralled those obtained at the NIMH Addiction Research Center in Lexington Kentucky. In both these centers, investigations of the characteristics of the withdrawal syndrome in man and animals have led to the recognition of the fact that it has features that are common to most species, regardless of the identity of the addicting drug. At the same time, research at both centers has made it possible to measure quantitatively the intensity and duration of the

abstinence illness. It is now possible to make a preliminary estimate based on work in primates that has relevance to the risk of abuse of and addiction to that drug in man.

It is obvious that subjective changes produced by drugs can only be measured in man, although one gets a fairly reliable idea of some of the subjective phenomena in animals by simply observing them carefully. Anyone who has worked with mescaline in the dog or monkey knows that these animals are hallucinating, but, since there is no way of proving it, most of us don't have the courage of our convictions and would never put such statements in a scientific paper. Despite this limitation, it is still possible to qualitatively describe and quantitate the abstinence syndrome in animals, especially the monkey, using objective criteria very similar to those devised for the assessment of this syndrome in man.

Table I shows the large number of signs that are used in a weighted fashion in order to arrive at a score that gives one an idea of the intensity of the syndrome in the monkey. It is a close approximation of the Himmelsbach point system devised in Lexington,[26] the most important difference being that subjective signs such as nausea, muscular discomfort and pain, burning sensations, abdominal cramps, dizziness, headache and so on are omitted. In the monkey, it is possible to chart the temporal course of withdrawal and to measure the intensity, the peak and the duration of the syndrome with precision and great reliability. The overall point scores are compiled for each drug and some estimate can be made of its addiction potential in man. Of course, there are some differences between monkey and man in this respect but for the most part there is a striking parallelism.

TABLE I.—*Signs of Abstinence in the Monkey*

Gastro-Intestinal	Behavior (cont.)
Retching	Fighting
Vomiting	Masturbation
Diarrhea	Lying on Side
Anorexia	Peculiar Postures
	Insomnia
Central Nervous System	Holding Abdomen
Muscle Rigidity	Calling and Crying
Muscle Twitches	Docility
Body Tremors	Prostration
Convulsions	
	Autonomic Nervous System
Metabolism	Yawning
Peak Respirations/Minute	Lacrimation
Lowest Temperature	Rhinorrhea
Weight Loss (Kg)	Sweating Face
Dyspnea	Piloerection
	Miosis
Behavior	Pallor
Apprehension	Conjunctivitis
Restlessness	Vascular Collapse
Chattering	
Quarreling	Death

Once the criteria for the measurement of dependence were worked out for the monkey, they were used in two general procedures developed by Irwin[27] and by Deneau and Seevers.[28] The first, the single-dose suppression test, ascertains whether the test drug is capable of complete suppression of all abstinence signs in the morphine-dependent monkey. A large colony of monkeys is maintained in a state of morphine dependence and when a drug under study is to be tested, the morphine injections are discontinued until abstinence signs appear. The test drug is then substituted for morphine and the suppression or lack of suppression of abstinence is noted. The second procedure is a direct addiction test, in which the monkeys are chronically treated with the test drugs for a period of several weeks, the drug then withdrawn and the severity of abstinence compared with that of morphine-dependent animals.

Although studies in primates have proven to be extremely valuable, there are obvious drawbacks. Because of the cost of maintaining a primate colony, the difficulty of working with large and often extremely aggressive animals, and the expertise needed to evaluate the signs of abstinence, a number of smaller and/or less expensive species have been evaluated with reference to their usefulness in the assessment of addiction potential.

The mouse has been used by a number of investigators but, in general, proves to be less satisfactory than other species. Tolerance is difficult to induce and the precipitation of abstinence by the antagonists is subject to variability due to strain, age and method of drug administration. Recently two Chilean investigators[29] developed a method of pellet implantation of morphine in mice and have been able to induce both tolerance and physical dependence in the implanted animals. Way and his associates have devised a test for dependence in which antagonist-treated mice are allowed to

jump from a circular platform—a high laboratory stool.[30] The number of animals jumping is used as a quantal measure of the degree of dependence. No analogous test has as yet been devised for man.

Shemano and Wendel[31] have proposed that the Straub reaction be used as a rapid screening test for the potential addiction liability of new analgesics. They have proposed a use of the ratio of the CD_{50} to the Straub ED_{50}, the "Straub-index", as a predictor of human addiction liability; it seems to work quite well.

The rat has been used for many years as one of the preferred species in which to study both the development of tolerance and physical dependence and the abstinence syndrome has been described in almost as much detail as in the monkey. A simple discriminatory training test has been used to show both tolerance and abstinence in the rat,[32] and the shaking behavior seen in abstinence, called "wet dog" shakes, has been proposed as a quantitative measure of abstinence severity.[33] Although morphine-like drugs induce a somewhat different profile of effects in the rat than in man, the rat is nevertheless an eminently satisfactory test animal for the assessment of dependence liability. It is much easier to use than the monkey, more feasible for early screening, and the parallelism between rat and man is not much less than between monkey and man.

The cat presents some difficulties, since it, as well as the tiger and the lion, reacts to morphine with a profile of stimulatory behavior. The pattern is consistent, however, and is reversed by the antagonists. It has not been widely used—the mere sight of a cat after a morphine injection is enough to make most investigators turn to other species for their studies.

A large number of investigations of tolerance and physical dependence have been carried out with dogs, and methods for the estimation of dependence in both the intact and spinal dog have been developed at the NIMH Addiction Research Center.[34,35] These techniques involve infusions of morphine for 8 hours and precipitation of abstinence by an antagonist. They may have some usefulness as preliminary screening techniques. Chronic spinal dogs also exhibit a stereotyped withdrawal and have been used to screen new analgesics over long periods of time. There are obvious difficulties in preparing and maintaining spinal preparations and this has discouraged widespread use.

EVALUATION OF PSYCHOLOGICAL DEPENDENCE

Although it had long been thought that the measurement of subjective drives in animals was not an attainable goal in the forseeable future, recent developments have not only made it possible but practical. The development of operant-conditioning techniques and the adaptations of these techniques to the problems of self administration of drugs by animals have enabled investigators to address themselves to the study of the phenomenon of psychological dependence —drug-seeking behavior.

One of the simplest animal models for drug-seeking behavior was one proposed years ago by Spragg,[36] a psychologist working at the Yerkes Primate Center. In the course of a study of addtion in chimpanzees, he noticed that his subjects showed purposive drug-seeking behavior after a long period of morphine administration. They would clamor to be taken from the cages to the injection room and clearly preferred the syringe to food when they were both abstinent and hungry, whereas shortly after an injection they chose food. While this study showed that the relief of abstinence is a sufficient reinforcement for the drug drive in the primate, the question of euphoria as a reinforcement in animals remains unanswered. However, the findings of Tatum and Seevers[37] that dogs develop cocaine-seeking behavior is evidence that the drug itself serves as a reinforcement since no physical dependence develops with cocaine. A number of investigators have looked at the elective drinking of water or drug solutions by chronically morphinized rats as a simple model of drug-seeking behavior, but the aversive taste of narcotic solutions has confounded the results in most cases.

In 1962 Weeks[38,39] developed a most ingenious and practical technique for self administration of drugs that has stimulated a large number of studies in the rat and the monkey.

This technique, utilizing operant-conditioning procedures by which the animal can control the frequency and to some extent the amount of drug it receives, has been an extremely important advance in methodology. A cannula is inserted into the right atrium of the rat or monkey via the jugular vein and connected to a syringe driven through a swivel arrangement that allows free movement. An infusion pump can deliver a programmed series of injections automatically or it can be activated by a bar that the monkey presses. The animal can adjust the frequency of the lever presses until he allays his drug hunger or his abstinence symptoms. If the experimenter reduces the concentration of the drug and thus the amount, the animal will increase the frequency of bar presses if it is dependent. The schedules, the ratio of bar presses to drug infusion and the interval between rewards can be adjusted as the experimenter wishes. There seems to be no doubt that animals work for morphine reward and that they will self administer drugs such as cocaine and amphetamine where physical dependence cannot be an important factor.[40] The importance of these experiments is difficult to overestimate. There seems to be no doubt that the monkey closely resembles man in its liking for and dislike of drugs. The major drugs that man abuses—morphine, the barbiturates, cocaine amphetamines, alcohol and codeine—have also been shown to be abused by the monkeys.

SUMMARY

There is no question that these procedures will permit investigators to study, assess and evaluate many facets of the phenomenon of addiction in animals which we were not able to do previously. It is quite possible that the importance of evaluation of subjective drug effects will decrease as such animal techniques become more refined and sophisticated and this is, after all, what animal models are for—to avoid doing in man what can be done in animals and to help us to understand what happens in man.

REFERENCES

1. Halbach, H., and Eddy, N. B. Tests for addition (chronic intoxication) of morphine type. Bull. World Health Organ. 28:139, 1963.
2. Collier, H. O. J. Analgesics: Evaluation of Drug Activities, Pharmacometrics, Vol. 1. (D. R. Laurence and A. L. Bacharach, eds.), Academic Press, New York, 1964, p. 183.
3. Winter, C. A. The physiology and pharmacology of pain and its relief. Analgesics (G. De Stevens, Ed.), Academic Press, New York, 1965, p. 9.
4. D'Amour, F. E., and Smith, D. L. Method for determining loss of pain sensation. J. Pharmacol. Exptl. Therap. 72:74, 1941.
5. Grotto, M., and Sulman, F. G. Modified receptacle method for animal analgesimetry. Arch. Intern. Pharmacodyn 165:152, 1967.
6. Andrews, H. L., and Workman, W. Pain threshold measurements in dog. J. Pharmacol. Exptl. Therap. 73:99, 1941.
7. Winder, C. V. Some examples of the use of statistics in pharmacology. Ann. N.Y. Acad. Sci. 52:838, 1950.
8. Hardy, J. D., Wolff, H. G., and Goodell, H. Studies on pain. New method for measuring pain threshold: observations on spatial summation of pain. J. Clin. Invest. 19:649, 1940.
9. Eddy, N. B., and Leimbach, D. Synthetic analgesics, dithienylbutenyl and dithienylbutylamines. J. Pharmacol. Exptl. Therap. 107:385, 1953.
10. Cochin, J., and Kornetsky, C. Development and loss of tolerance to morphine in the rat after single and multiple injections. J. Pharmacol. Exptl. Therap. 145:1, 1964.
11. Cochin, J., and Kornetsky, C. Factors in blood of morphine-tolerant animals that attenuate or enhance effects of morphine in non-tolerant animals, Chapter XX. The Addictive States, Vol. XLVI. Association for Research in Nervous and Mental Diseases. (Wikler, A. ed.) Williams and Wilkins Co., Baltimore, pp. 268–279, 1968.
12. Haffner, F. Experimentalle prufung schmerzstellender mittel. Deut. Med. Wochschr. 55:731, 1929.
13. Eddy, N. B. Studies of morphine, codeine and their derivatives; general methods. J. Pharmacol. Exptl. Therap. 45:339, 1932.
14. Randall, L. O., and Selitto, J. J. A method for measurement of analgesic activity on inflamed tissue. Arch. Intern. Pharmacodyn. 111:409, 1957.
15. Siegmund, E., Cadmus, R., and Lu, G. A method for evaluating both non-narcotic and narcotic analgesics. Proc. Soc. Exptl. Biol. Med. 95:729, 1957.
16. Koster, R., Anderson, M. and De Beer, E. J. Acetic acid for analgesic screening. Federation Proc. 18:412, 1959.
17. Emele, J. F., and Shanamán, J. Bradykinin writhing: a method for measuring analgesia. Proc. Soc. Exptl. Biol. Med. 114:680, 1963.
18. Eckhardt, E. T., Chaplovitz, F., Lipo, M. and Govier, W. M. Etiology of chemically induced writhing in mouse and rat. Proc. Soc. Exptl. Biol. Med. 98:186, 1958.
19. Evans, W. O., and Bergner, D. P. A comparison of the analgesic potencies of morphine, pentazocine

and a mixture of methamphetamine and pentazocine in the rat. J. New Drugs 4:82, 1964.

20. Hill, H. E., Belleville, R. E., and Wikler, A. Reduction of pain-conditioned anxiety by analgesic doses of morphine in rats. Proc. Soc. Exptl. Biol. Med. 86:881, 1954.
21. Weiss, B., and Laties, V. G. Fractional escape and avoidance on a titration schedule. Science 128:1575, 1958.
22. Weiss, B., and Laties, V. G. Titration behavior on various fractional escape programs. J. Exptl. Anal. Behavior 2:227, 1959.
23. Weiss, B., and Laties, V. G. Changes in pain tolerance and other behavior produced by salicylates. J. Pharmacol. Exptl. Therap. 131:120, 1961.
24. Kornetsky, C., and Bain, G. Single dose tolerance to morphine. The Pharmacologist 9:197, 1967.
25. Weiss, B., and Laties, V. G. Analgesic effects in monkeys of morphine, nalorphine and a benzomorphan narcotic antagonist. J. Pharmacol. Exptl. Therap. 143:169, 1964.
26. Himmelsbach, C. K. Studies of certain addiction characteristics of dihydromorphine ("paramorphan"), dihydrodesoxymorphine -D ("desomorphine"), dihydrodesoxycodeine -D ("descodeine") and methyldihydromorphinone ("metapon"). Journal of Pharm. and Exptl. Therap. 67:239, 1939.
27. Irwin, S., Characteristics of depression, antagonism, and development of tolerance, physical dependence and neuropathology to morphine and morphine-like agents in the monkey (macaca mulatta). Dissertation Abstr. 14:686, 1954.
28. Deneau, G. A., and Seevers, M. H. Evaluation of Drug Activities: Pharmakometrics, Chapter 7. (Laurence, D. R. and Bacharach, A. L., eds.) Vol. 1. Academic Press, New York, 167–179, 1964.
29. Maggiolo, C., and Huidobro, F. Administration of pellets of morphine to mice; abstinence syndrome. Act Physiol. Latinoamer. 11:70, 1961.
30. Way, E. L., Loh, H. H., and Shen, F. Morphine tolerance, physical dependence and synthesis of brain 5-hydroxytryptamine. Science 162:1290, 1968.
31. Shemano, I., and Wendel, H. A rapid screening test for potential addiction liability of new analgesic agents. Toxicol. Appl. Pharmacol. 6:334, 1964.
32. Mercier, J., and Mercier, E. Determination of addiction to morphine and of the toxic reactions to withdrawal by a psycho-physiological method. Ann. Pharm. Franc. 12:458, 1954.
33. Martin, W. R., Wikler, A., Eades, C. G., and Pescor, F. T. Tolerance to and physical dependence on morphine in rats. Psychopharmacologia 4:247, 1963.
34. Martin, W. R., and Eades, C. G. Demonstration of tolerance and physical dependence in the dog following a short-term infusion of morphine. J. Pharmacol. Exptl. Therap. 133:262, 1961.
35. Martin, W. R., and Eades, C. G. A comparison between acute and chronic physical dependence in the chronic spinal dog. J. Pharmacol. Exptl. Therap. 146:385, 1964.
36. Spragg, S. D. S. Morphine addiction in chimpanzees. Comp. Psycho. Monogr. 15:1, 1940.
37. Tatum, A. L., and Seevers, M. H. Experimental cocaine addiction. J. Pharmacol. Exptl. Therap. 36:401, 1929.
38. Weeks, J. R. Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. Science 138:143, 1962.
39. Weeks, J. R. Experimental narcotic addiction. Sci. Am. 210:46, 1964.
40. Deneau, G. A., Yanagita, T., and Seevers, M. H. Psychic Dependence Studies by Self-Administration Techniques in the Rhesus Monkey—Appendix 21. Minutes of the 27th meeting—Committee on Problems of Drug Dependence NAS-NRC, 4267–4269, 1965.

DISCUSSION

Chairman Cohen: I'd like to ask Dr. Cochin if there are any specific EEG effects that one can monitor in an animal, such as a monkey or a dog, to indicate whether or not there is any hallucinogenic action going on after the administration of, say, mescaline?

Dr. Cochin: In some work which is provocative but not conclusive. There are EEG changes with other central-reacting drugs that one can monitor even in the rat; with morphine, for instance, and the antagonists. With mescaline, EEG changes in the dog are really suggestive, but I think this is a field that should be explored because, for those of us who have worked with the drug, there is no question that something has happened to those animals that can only be described as an hallucinatory experience. They look and jump at imaginary objects. They exhibit all sorts of bizarre behavior.

PHARMACOLOGIC ACTIONS OF NEWER BETA BLOCKING AGENTS

Lawrence S. Cohen,* George F. Vastagh** and Jere H. Mitchell**

ABSTRACT

Cardiac beta-adrenergic receptor blockade decreases chronotropic and inotropic responses. In an open chest canine preparation in which aortic pressure, cardiac output, and heart rate could be controlled, the chronotropic and inotropic effects of Propranolol (P), MJ–1999 (Sotalol) (S), AY21011 (Practolol) (AY), and Alprenolol (A) were studied before and after isoproterenol challenge (I). Equiactive doses of these agents were compared based on initial studies which determined the ability to block an I.

In unpaced studies S>P>AY=A caused a dose dependent negative chronotropic effect. After I, AY>P>A>S progressively blocked the positive chronotropic effect as the amount of blocker was increased.

In paced studies all 4 agents lowered left ventricular dp/dt equivalently. After I, AY>P=S=A progressively blocked the inotropic effect.

These data show that AY blocked the chronotropic and inotropic responses to I most effectively while having less intrinsic negative chronotropic activity. S had the greatest intrinsic negative chronotropic response and blocked the chronotropic response to I least. All 4 agents displayed equivalent intrinsic negative inotropic activity.

INTRODUCTION

In 1958 Powell and Slater introduced the first of the clinically useful beta adrenergic blocking agents, dichloroisoproterenol. Although this agent had potent beta blocking activity, it also possessed an intrinsic sympathomimetic action. Pronethalol, introduced in 1962, had little or no intrinsic sympathomimetic activity but was found to have a tumorigenic effect in mice. Propranolol, a more recent beta blocking agent, has been investigated intensively and has been utilized clinically in a variety of cardiac disorders, particularly angina pectoris. One of the limitations in the use of propranolol has been a direct myocardial depressant action separate from its beta adrenergic blocking action. Recently several newer beta blocking agents, reportedly with more cardiospecificity, have been introduced. MJ–1999 (Sotalol), Alprenolol (Aptine) and AY 21011 (Practolol) are three such agents. The object of this investigation was to compare the relative negative chronotropic and inotropic properties of Propranolol, Sotalol, Alprenolol and Practolol and to determine if there was any difference in their ability to induce beta adrenergic blockade to the chronotropic and inotropic properties of isoproterenol.

MATERIALS AND METHODS

In an open chest canine preparation, the brachiocephalic, left subclavican and upper intercostal arteries were ligated and the descending aorta cannulated. The left ventricle pumped blood into a blood-primed extra corporeal circuit from which blood was returned to the descending aorta and carotid arteries. The extra corporeal system included a bottle of adjustable height which held aortic pressure constant, a rotor pump which returned a constant flow to the dog, and a heat exchange unit which maintained a constant blood temperature. An electrode was sutured to the right atrium and connected to a Grass impulse generator for control of heart rate. Pressures were recorded from 3 Statham P23Db strain gauge transducers; one attached to a wide-bore cannula which was placed into the left ventricular cavity through an apical dimple, one into the arch of the aorta through a carotid artery, and one into the tubing carrying blood from the extra corporeal pump into the body. An R–C electronic circuit continuously differentiated the signal from a Dallons-Telco catheter tip manometer in the left ventricular cavity. In this preparation, the

* Yale University School of Medicine, New Haven, Connecticut.
** The University of Texas (Southwestern) Medical School at Dallas, Dallas, Texas.

cardiac beta adrenergic blocking actions of a drug upon the heart may be studied exclusive of the effects of beta blockade upon the peripheral vascular system.

The experimental design of the study appears in Figure 1. In the control state the initial heart rate and the chronotropic response to an isoproterenol challenge was determined. Similarly, the left ventricular dp/dt during atrial pacing was measured and the left ventricular dp/dt response to an isoproterenol challenge during atrial pacing was measured.

Initially for each beta blocker investigated, the dose of drug necessary to completely inhibit the positive chronotropic and inotropic response to an isoproterenol challenge was determined. This dose of beta blocker was considered 100% blockade. Five serial dilutions were then made so that the first concentration utilized was 3⅛% of the total blocking dose.

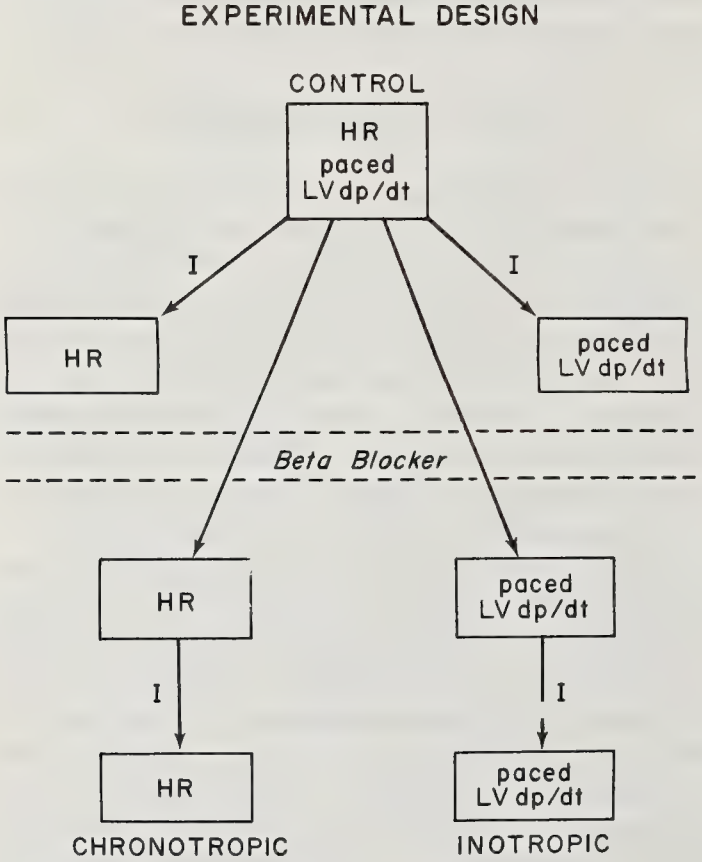


FIGURE 1.—Experimental design of study. In the control state initial heart rate and the chronotropic response to an isoproterenol challenge was determined. Similarly the left ventricular dp/dt during atrial pacing was measured and the left ventricular dp/dt response to an isoproterenol challenge during atrial pacing was measured.

After injection of the lowest concentration of drug, heart rate was measured, as was the heart rate response to an isoproterenol challenge. At each concentration of beta blocker injected the heart rate changes due to beta blocker alone and the attenuation of isoproterenol response could then be determined. Pacing was then instituted and left ventricular dp/dt was measured as was the left ventricular dp/dt response to isoproterenol. The intrinsic left ventricular dp/dt response to beta blockade and the attenuation of isoproterenol response could then be determined.

These studies were then repeated at increasing concentrations of beta blocker. No animal received more than 1 beta blocking agent and six animals received each beta blocking drug. Measurements of heart rate and dp/dt were made one minute after injection of drug into the inferior vena cava.

RESULTS

A representative tracing appears on Figure 2. Left ventricular dp/dt appears on the top. Aortic pressure (AP) and left ventricular pressure (LVP) are measured in mm.Hg. Left ventricular diastolic pressure is measured in cm. H_2O. The time is recorded on the abscissa.

The data in Figure 3 and the three to follow represent six animal experiments for each drug. The dose of drug administered, in mg/kg intravenously, appears on the bottom of the figure. Equi-potency was judged by the dose necessary to completely block the chronotropic and inotropic response to isoproterenol. A single star denotes the level of significance from control of a paired Student's T test at the 0.05 level and a double star denotes significance at the 0.01 level. The intrinsic chronotropic changes of beta blockade are plotted on this slide. On the ordinate is the percent change from control and on the abscissa is the dose of drug and the percent of total blocking dose.

Sotalol>Propranolol>AY 21011 = to Alprenolol caused a dose dependent negative chronotropic response. The change in heart rate com-

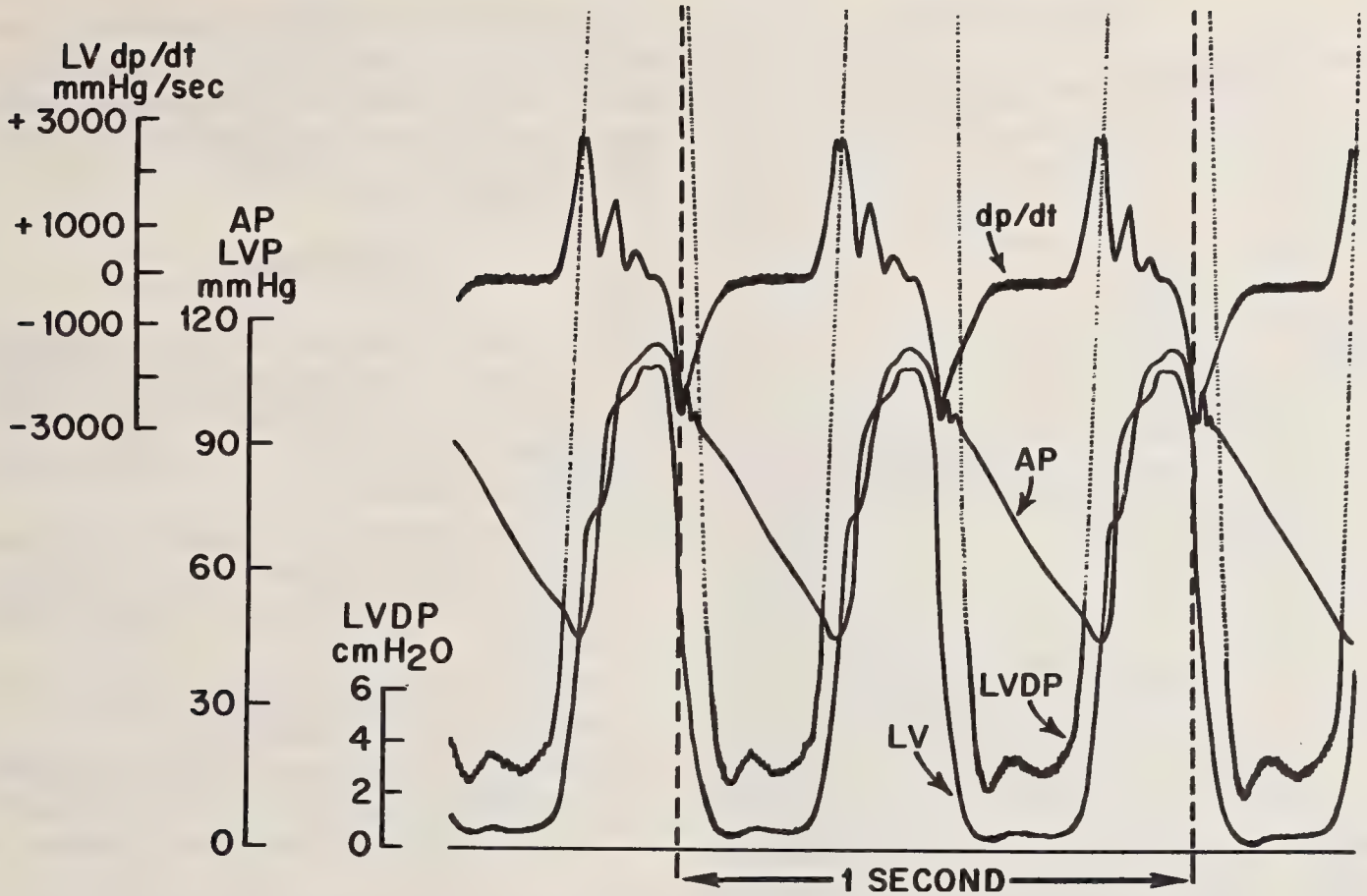


FIGURE 2.—Representative tracing of experimental measurements. Left ventricular dp/dt appears at the top. Aortic pressure (AP) and left ventricular pressure (LVP) are measured in mm.Hg. Left ventricular diastolic pressure is measured in cm.H_2O. The time is recorded on the abscissa.

pared with control values was significant only at high doses of Sotalol and propranolol.

The four agents displayed a differing ability to block the chronotropic response to an isoproterenol challenge (Figure 4). On the ordinate, 100% represents the change from control heart rate following an isoproterenol challenge. The response to isoproterenol following increasing doses of beta blocker is plotted as a percent of the response to isoproterenol prior to beta blockade. AY 21011 blocked the chronotropic response to isoproterenol most effectively followed by propranolol, aptine and sotalol.

In a paced preparation, the intrinsic effects of beta blockade upon left ventricular dp/dt were investigated (Figure 5). All four agents lowered intrinsic left ventricular dp/dt equivalently.

The left ventricular dp/dt response to an isoproterenol challenge was then looked at (Figure 6). AY 21011>Propranolol>Sotalol = Alprenolol blocked the inotropic response to isoproterenol in that order.

Based on these data it would appear that:

1. AY 21011 (Practolol) blocked the chronotropic and inotropic responses to isoproterenol most effectively, followed closely by propranolol.
2. All four agents displayed equivalent intrinsic negative inotropic activity.
3. Sotalol had the greatest intrinsic negative chronotropic effect followed by propranolol.

DISCUSSION

It is difficult to extrapolate these findings from the animal laboratory to the clinical situation of angina pectoris. It is agreed generally that angina pectoris reflects a dynamic state in which myocardial oxygen requirements exceed myocardial oxygen supply. The major deter-

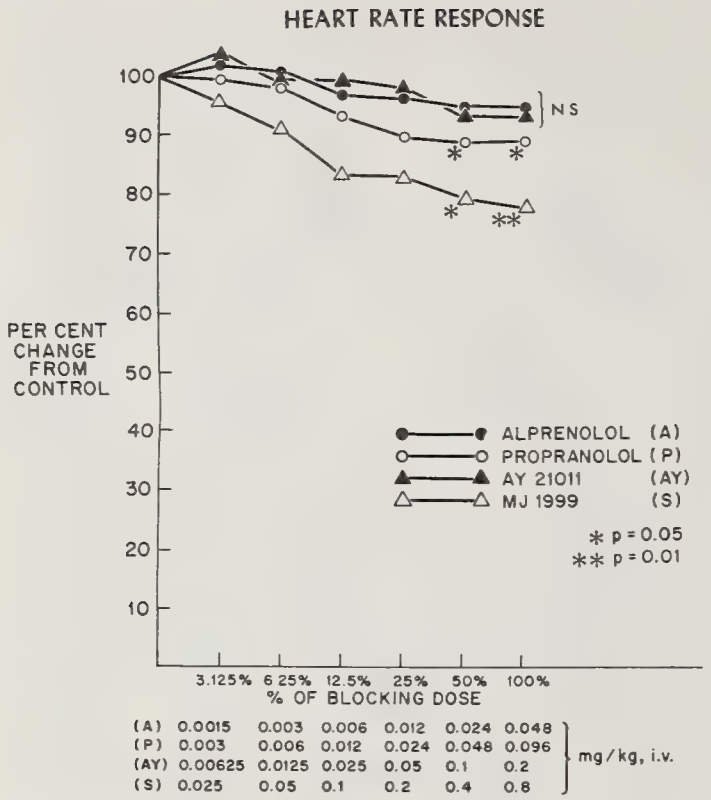


FIGURE 3.—Heart rate response to increasing doses of drug. Sotalol>Propranolol>AY21011=Alprenolol caused a dose dependent negative chronotropic response.

minants of oxygen need are heart rate, velocity of contraction and developed tension. Developed tension is in part related to end diastolic volume due to the dependence of wall tension upon the radius of the left ventricle. The effectiveness of propranolol in anginal states seems to be related to its ability to inhibit the myocardial responses to exercise induced catechol stimulation. Nevertheless, based on this investigation AY 21011 (Practolol) deserves further study as a possible anti-anginal agent. Its ability at low concentrations to block both the chronotropic and inotropic properties of isoproterenol might mean a diminution in myocardial oxygen requirements by decreasing two of the major determinants of oxygen consumption; heart rate and velocity of contraction. Support for this hypothesis has come from a parallel study of these agents in the exercise laboratory. In a group of patients with established ischemic heart disease, studied over a 1–2 year period, the average increase in physical performance with placebo was 3%; with Sotalol 21%, with propranolol 28% and with Practolol 38%. Based on these data, further clinical trials utilizing newer more specific beta adrenergic blocking agents for the control of angina pectoris appear to be warranted.

SUMMARY

1. In an open chest canine preparation, the chronotropic and inotropic effects of Propranolol (P), MJ–1999 (Sotalol) (S), AY21011 (Practolol) (AY), and Alprenolol (A) were studied.
2. AY21011 (Practolol) blocked the chronotropic and inotropic responses to isoproterenol most effectively, followed closely by propranolol.
3. All four agents displayed equivalent intrinsic negative inotropic activity.
4. Sotalol had the greatest intrinsic negative chronotropic effect followed by propranolol.
5. The differing chronotropic and inotropic ef-

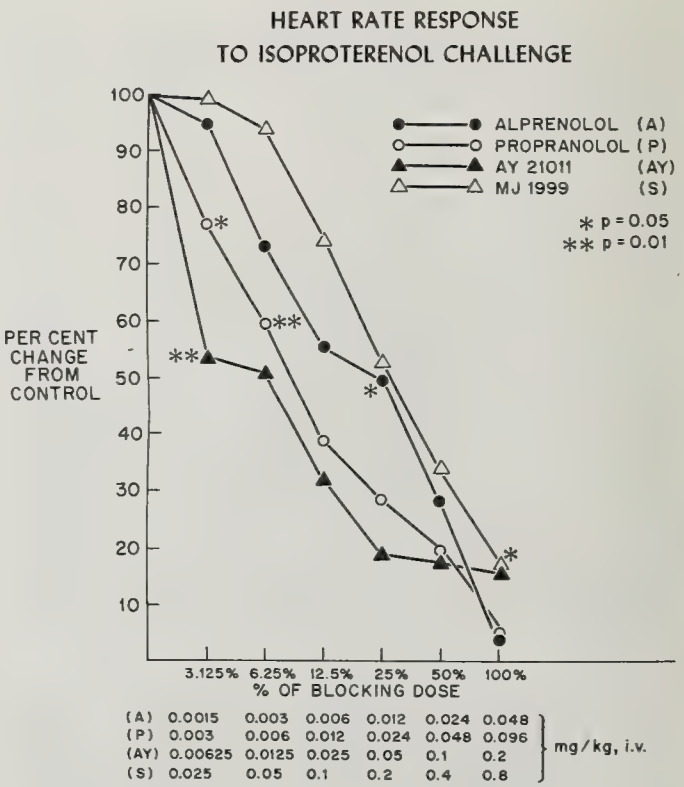


FIGURE 4.—The effect of beta adrenergic blockade upon the chronotropic action of isoproterenol. AY21011 blocked the chronotropic response to isoproterenol most effectively followed by propranolol, alprenolol and sotalol.

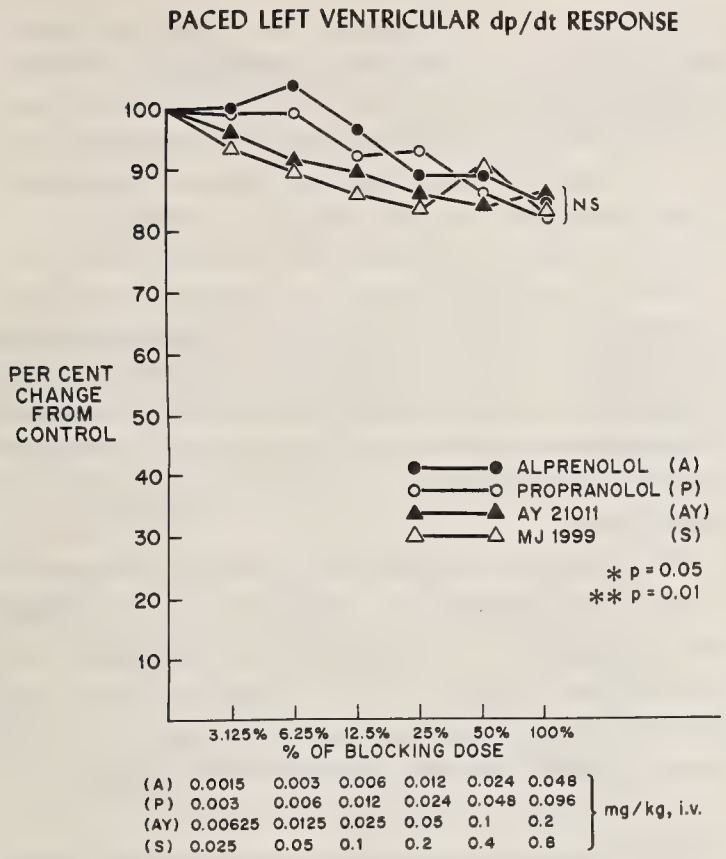


FIGURE 5.—During cardiac pacing the intrinsic effects of beta blockade upon left ventricular dp/dt. All four agents lowered intrinsic left ventricular dp/dt equivalently.

fects of these agents may prove to be significant in the treatment of patients with angina pectoris.

DISCUSSION

PITAMBAR SOMANI, Abbott Laboratories North Chicago: This was a very elegant study demonstrating the differences between various beta blockers. Some of the data that you have shown is at some variance with what other investigators have reported regarding the potency of practolol, because practolol in isolated systems, as well as in the whole animal is about $\frac{1}{10}$th as potent as propranolol in the same milligram dose. I was wondering if you would care to comment on this?

DR. COHEN: That is an excellent question, and one to which we address ourselves in the experimental design of the study. It became quite clear that one really cannot look at milligram to milligram doses of two drugs, and assume that they are of equal potency because they are the same milligram dosage. It is for that reason that we determined, for each of the four agents used, what was 100% blockade, and we defined *that* as a lack of response to an isoproterenol challenge. Now for one drug, just to give you figures, that dose might be 1 mg. For another drug, it might be 10 mg. We would consider them as being of equal potency, and that is 100% blockade by our criterion. At that point we use serial dilutions downwards. And so we did not use the same dose of drugs at each stage of the experimental design but we did use the same per cent of total blocking ability. I think in terms of our own study, this may answer some of the questions about "potency of different agents." Potency in and of itself really does not mean very much. It is pharmacologic potency that one wants to deal with.

DR. SOMANI: The second comment I had was in relation to the mechanism of anti-anginal

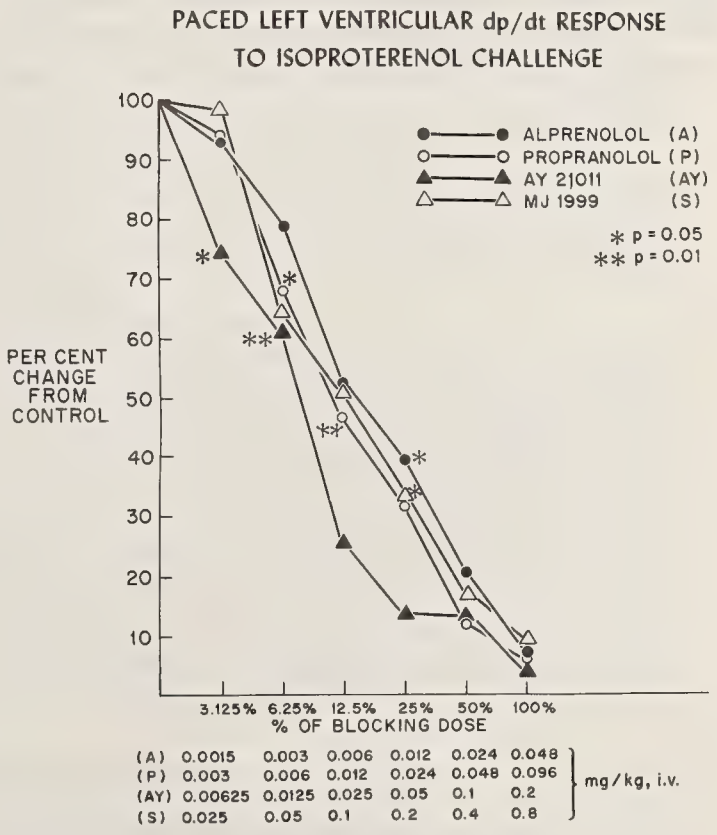


FIGURE 6.—Left ventricular dp/dt response to an isoproterenol challenge during cardiac pacing. AY210-11>Propranolol>Sotalol=Alprenolol blocked the inotropic response to isoproterenol in that order.

activity. Practolol has been shown clinically to reduce the incidence of anginal attacks and I think one important distinction between the two compounds, practolol and propranolol, would be their effects on coronary circulation. Propranolol blocks both beta I and beta II receptors. That means it would block the vasodilator effects of isoproterenol and thereby it would increase the coronary resistance. Practolol has no beta II blocking activity and therefore does not increase the coronary resistance. That is one distinction. However, in terms of anti-anginal activity, increase in total coronary resistance may not be important because the most important point would be redistribution of blood flow to the ischemic area. In this situation we have shown, and Burt Pitt has shown, that both practolol and propranolol would redistribute blood to the ischemic area. I think that is another important consideration for mechanisms of anti-anginal activity.

DR. COHEN: I am glad you mentioned your experiments, along with those of Dr. Pitt. It is quite clear that angina is a clinical situation where there is a disparity between myocardial oxygen consumption and myocardial oxygen needs. Any given drug may have varying levels of activity. I think most investigators, myself included, would say that the major locus of action of the beta adrenergic blocking agents was to cut down the myocardial oxygen consumption. A minor action may be redistribution, thereby making oxygen more accessible to ischemic areas. That is a fine distinction and one that should be borne in mind. At the same time, I think we would be making a mistake if we looked upon the beta blocking agents in the same way that we looked upon nitroglycerine, where we feel its major action is on coronary vascular resistance.

FRANCIS CAVOTO, Medical College of Pennsylvania, Philadelphia, Pa.: I have been interested in the effects of these drugs on sympathetic innervation. I know that MJ 1999 is not a good neural depressant whereas propranolol is. I was wondering what the relative neural depressant abilities of practolol are?

DR. COHEN: I am sorry. I cannot answer that. We did not do any direct studies of these drugs on neural activity. We addressed ourselves only to the cardiovascular effects.

COMPARATIVE PHARMACOKINETICS IN DOMESTICATED ANIMALS

L. E. Davis, C. H. Davis and J. D. Baggot*

We studied a series of drugs, selected on the basis of their physico-chemical properties, in ponies, goats, swine, dogs and cats. The drugs included five acidic drugs (salicylate, pentobarbital, phenylbutazone, oxyphenylbutazone and phenol) and five basic drugs (pentazocine, chloramphenicol, quinidine, amphetamine and tetraethylammonium). Parameters measured or computed included: elimination rate constants, drug concentrations in plasma, volumes of distribution and extent of drug-plasma protein binding. Drug absorption from depot sites following oral or intramuscular administration was investigated for some of the drugs. Some patterns in species differences were evident in our investigations. Drugs which were eliminated as conjugates with glucuronic acid, sulfate or glycine were eliminated from the body rapidly in herbivorous animals and slowly in carnivorous animals; omnivorous animals were intermediate. The cat was particularly handicapped in its ability to eliminate such compounds. Patterns were not predictable for drugs which were transformed by oxidative mechanisms. Specific volume of distribution for individual drugs was largest in ruminant animals and was much larger for basic drugs than for acidic drugs. In general, rates of elimination were not related to extent of drug-protein interaction, although species differences were observed in plasma protein binding of various drugs. From our experiments, we found marked species differences in the time course of drug concentrations in the plasma of representative large domesticated animals. This work illustrates the errors which may be encountered when one attempts to extrapolate information concerning drugs from one species to another.

INTRODUCTION

Relatively little is known regarding the time course of drug concentrations in the plasma of species other than man and the common laboratory animals. Such information is a necessary prerequisite for the determination of dosage regimens and interpreting experimental data. Brodie[1] indicated the need for information relative to species differences in the rate of metabolism of drugs. On the basis of the importance of this knowledge to the preclinical testing of new drugs, it has been suggested that a search should be made for animal species which metabolize given classes of compounds at the same rate as does man.[2,3] Knowledge of the disposition and fate of drugs in large farm animals is extremely limited and systematic investigations of pharmacokinetics in these species have not been conducted. Accordingly we have studied the time-course of drug concentrations for several drugs in ponies, goats, swine, dogs and cats.

* Division of Comparative Pharmacology, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.

MATERIAL AND METHODS

Animals

The animals employed in these studies were sexually mature Shetland cross ponies, Hampshire and Yorkshire swine, Angora goats, mongrel dogs and American short haired cats of both sexes. All subjects were healthy at the time of the experiments.

During the experiments animals were confined to metabolism cages or crates. The particular drug of interest was administered and blood samples were collected at appropriate intervals. Urine samples were collected for 24 hours. Retention catheters were passed into the urinary bladders of mares, does and sows. Voided urine was collected from all other animals. All samples were analyzed immediately or frozen pending analysis.

Analytical Procedures

The concentrations of the various drugs were determined by established procedures as follows: salicylate,[4] pentobarbital,[5] phenylbutazone,[6] phenol,[7] pentazocine,[8] chloramphenicol,[9] quinidine,[10] and amphetamine.[11] Tetraethylam-

monium-^{14}C with carrier was administered and the drug concentrations in biological samples were determined by radioassay.

Protein Binding

The extent of plasma protein binding of the various drugs was determined by equilibrium dialysis. Isotonic phosphate buffer, pH 7.4, containing ^{14}C or ^{3}H labeled drug was dialysed against an equal volume of plasma for 16 hours at 37° C. Aliquots of plasma and buffer were assayed for radioactivity. Protein concentrations were measured by refractometry.

Calculations

Values for the Y-intercept (Co) of the curves depicting drug disappearance and the slopes of these curves were determined from the drug concentrations in plasma, as a function of time, by a procedure for fitting an exponential curve utilizing a Wang electronic calculator with card programmer. The computation of the kinetic constants was made with the equations described by Doluisio and Swintosky.[12]

RESULTS

Figure 1 shows the mode of plasma decay of salicylate in each of the species studied. Disappearance followed first-order kinetics throughout the entire period studied in ponies, goats and cats; and for the first eight hours in swine and dogs. The reason for the increased disappearance rate between 8 and 24 hours in the dog and pig was not apparent but was probably a manifestation of saturation kinetics.

Table I illustrates mean values for the kinetic constants for each of the species studied. A tremendous variation in rates of disappearance was noted among the species. Apparent specific

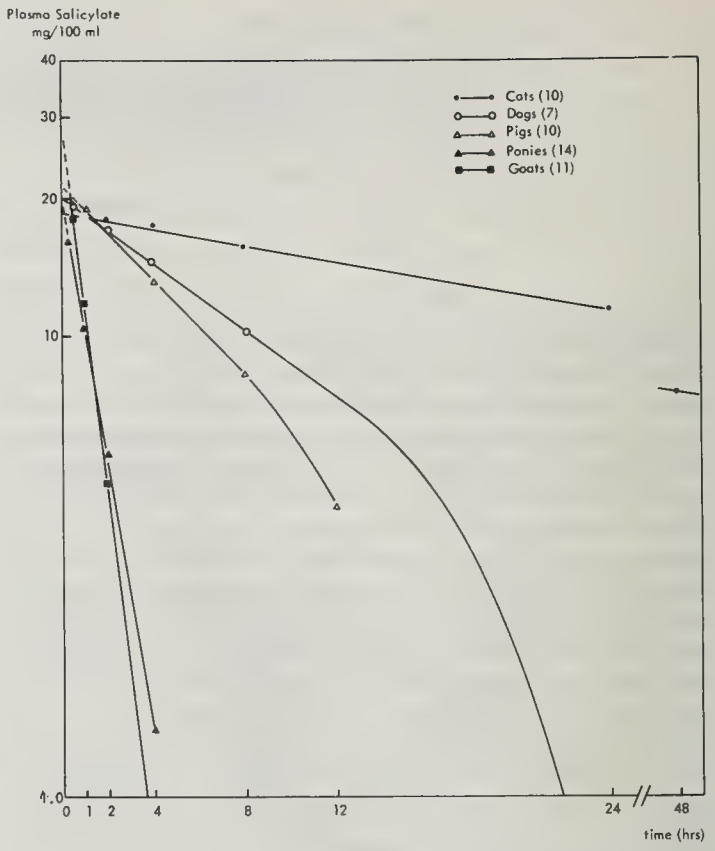


FIGURE 1.—Disappearance of salicylate from the blood plasma of several species following the intravenous injection of sodium salicylate (44 mg/kg). Each point represents the mean concentration and the number in parentheses is the number of animals studied.

volumes of distribution suggest that salicylate was confined largely to the extracellular space in the species studied. The low value for V'd in the goat is believed to illustrate the error in dosage introduced by the weight of the rumen and its contents. It is apparent from Co that the dose administered to goats produced an extraordinary plasma level as compared to the other species. The rumen and its contents comprise approximately 25% of the body weight and apparently were not included in the volume of dis-

TABLE I.—*Comparison of Kinetic Constants for the disappearance of Salicylate from the Blood Plasma of Several Species*

Species	No.	Mean Wt.	Mean Dose[a]	Co	Vd	V'd	kd	T½
		kg	mg	mg/100 ml	L	L/kg	hr^{-1}	hr
Cat	10	2.3	89.4	18.5	0.48	0.209	0.018	37.6
Dog	7	8.5	322	20.0	1.61	0.189	0.081	8.6
Swine	10	120.3	4551	21.3	21.37	0.178	0.118	5.9
Pony	14	197.0	7455	21.1	35.33	0.180	0.673	1.0
Goat	11	41.1	1555	29.3	5.31	0.129	0.884	0.8

[a] Calculated as the free acid. Co = plasma concentration of drug at zero time. Vd = apparent volume of distribution. V'd = apparent specific volume of distribution. Kd = apparent first-order disappearance rate constant. T ½ = half-life of drug in plasma.

tribution. It may be noted that the apparent specific volume of distribution of salicylate in the goat was approximately 75% that calculated for the pig and pony.

The herbivorous animals conjugated salicylate with glycine at a much more rapid rate than did the omnivorous pig or carnivorous dog. Rate of glucuronidation of salicylate was higher in the goat and dog than in the pony and pig. The species differences in rate of excretion of unchanged salicylate was related in part to urinary hydrogen ion concentration. The pH of goat urine averaged 8.2 and pony urine 7.6 while pig urine was 6.4 and dog urine 5.8. This observation is in agreement with the relationship between urinary pH and renal clearances of salicylate reported by Smith and co-workers in human patients.

The cat was handicapped in several ways in its ability to excrete salicylate. It was deficient in the ability to synthesize metabolites which are more polar than the parent compound and the urine was quite acid (pH 5.2) thereby decreasing renal clearance of the drug.

Data on the effects of chronic administration of the drug showed no changes in plasma half-life and the distribution of salicyl metabolites in urine remained constant over the six-month period in the control groups. The percentage of unchanged salicylate remained fairly constant in the low and therapeutic dose groups whereas the high dose groups excrete a larger percentage of salicylate after six months of chronic administration. The percentages of salicylglucuronide increased as a result of chronic administration and this increase was at the expense of salicylurate synthesis in all species. This would suggest the possible induction of glucuronyl transferase. No untoward effects of the drug were noted except in cats. Cumulation of the drug produced ulceration of the GI tract with hemorrhage. No changes were noted in the hemograms which could be attributed to the drug and no fetal anomolies were observed.

Absorption, following oral, administration, was found to be rapid and complete in all species except the goat. In this species we observed several absorption peaks in the plasma over the 12-hour periods studied. These probably are related to periodic overflow of rumen contents into the abomasum or true stomach. These data are shown in Figure 2. Plasma protein-binding was studied in each species, including man. These data are tabulated in Table II.

Phenol

Phenol and its synthesized metabolites, phenyl sulfate and phenylglucuronide, were characterized by physical properties and separated by chromatography. LD 50's were determined and revealed effective phenol detoxication by conjugation with sulfate and glucuronate. A

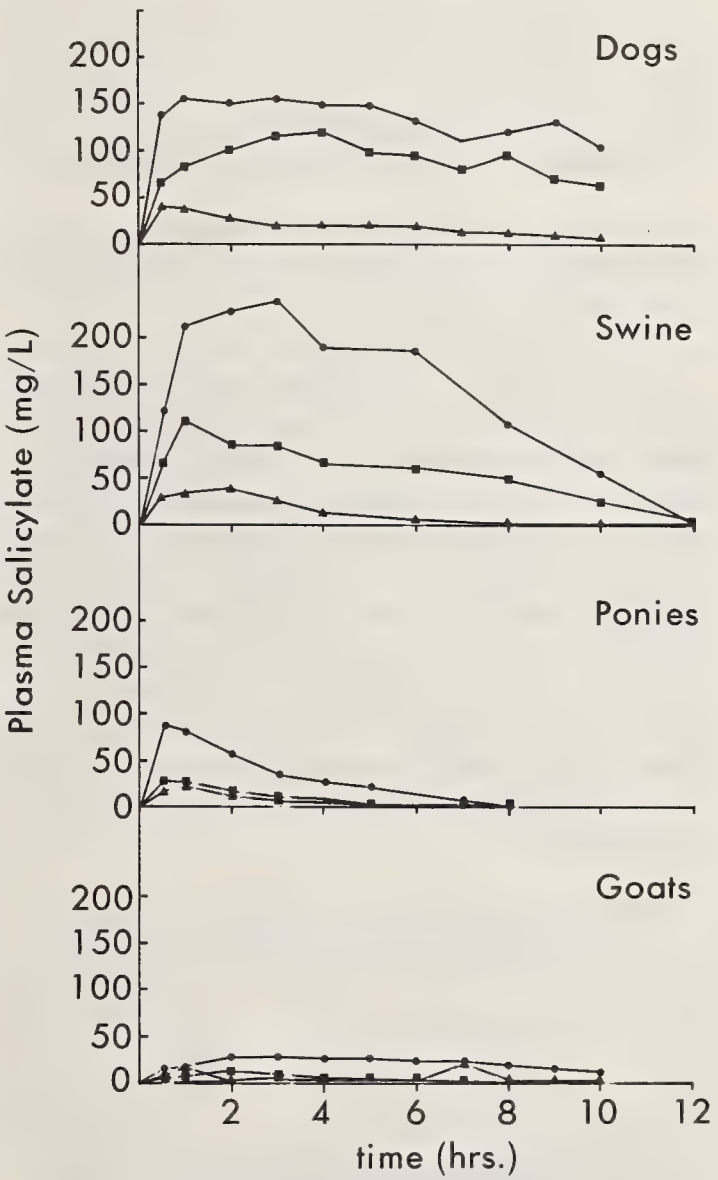


FIGURE 2.—Species differences in absorption of sodium salicylate following the oral administration of the drug contained in gelatin capsules. Three dosage levels (18.5, 50 and 133 mg/kg) were studied. The data points represent the means from at least four animals.

TABLE II.—*Species Differences in Plasma Protein Binding of Salicylate*[a]

Species	Plasma Protein Concentration	Percent Bound By Plasma Protein
	g/100 ml.	%
Human	6.3	85
	8.0	85
	7.9	38
Dog	6.7	70
	6.9	56
	7.2	53
Goat	6.4	64
	6.7	58
	6.6	63
Swine	6.3	64
	7.8	68
	7.9	72
Pony	6.8	54
	7.0	52
	6.7	57
Cat	6.5	54
	7.7	64
	7.9	60

[a] Three different individuals of each species were studied.

fluorometric procedure was developed to measure phenol in biological materials. This method was specific and sensitive.

Plasma disappearance and urinary excretion of intravenously administered phenol were studied in dogs, cats, swine and goats. Cats were acutely poisoned by dosages of 50 mg/kg, whereas dogs, pigs, and goats suffered sublethal intoxication from doses of 100 mg/kg. Plasma disappearance of injected phenol was most rapid in goats, slower in pigs and dogs, and slowest in cats (Figure 3, and Tables III–VI). The time required for most of the injected phenol to be in the conjugated form in the plasma was directly proportional to the rate of plasma disappearance and inversely related to the dose given.

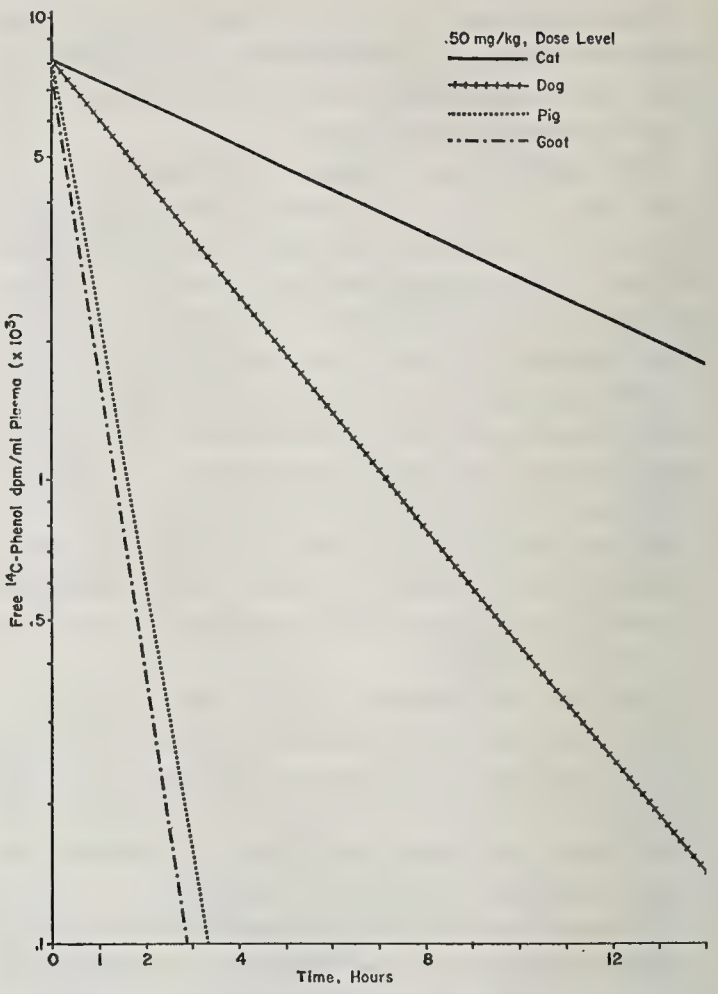


FIGURE 3.—Disappearance of injected free ^{14}C-phenol from the plasma of dogs, cats, swine and goats. The curves were adjusted slightly to a similar concentration at time zero.

The apparent specific volumes of distribution of free phenol in dogs, pigs and goats indicated extensive tissue binding of phenol. Such evidence for tissue binding was not observed in

TABLE III.—*The Kinetic Constants for Plasma Disappearance of Phenol in Dogs*

Dose	C_0	T½	Kd	V'd
mg./kg.	dpm x 10^3/ml.	hrs.	hr.$^{-1}$	L./kg.
10	4.2	1.36	0.51	0.41
50	5.9	2.56	0.27	1.59
100	9.3	1.70	0.41	2.08

TABLE IV.—*The Kinetic Constants for Plasma Disappearance of Phenol in Cats*

Dose	C_0	T½	Kd	V'd
mg./kg.	dpm x 10^3/ml.	hrs.	hr.$^{-1}$	L./kg.
10	3.9	1.22	0.57	0.47
30	9.9	1.67	0.42	0.19
50	8.6	7.00	0.10	0.19

TABLE V.—*The Kinetic Constants for Plasma Disappearance of Phenol in Pigs*

Dose	C_0	T½	Kd	V'd
mg./kg.	dpm x 10^3/ml.	hrs.	hr.$^{-1}$	L./kg.
10	0.8	0.84	0.83	2.40
50	6.6	0.54	1.28	1.53
100	15.8	0.48	1.44	1.72

TABLE VI.—*The Kinetic Constants for Plasma Disappearance of Phenol in Goats*

Dose	C_0	T½	Kd	V'd
mg./kg.	dpm x 10^3/ml.	hrs.	hr.$^{-1}$	L./kg.
10	2.4	0.44	1.58	0.79
50	8.8	0.48	1.43	1.09
100	14.8	0.51	1.36	1.28

cats. The extent of plasma protein binding of phenol was about 60% in all species. The total endogenous phenols excreted in the urine of normal animals in 24 hours was less than 0.5 mg/kg in dogs, slightly more than 2 mg/kg in cats, slightly more than 3 mg/kg in swine and 7.5 mg/kg in goats. Dog urine contained equal portions of free phenol, phenylsulfate and phenylglucuronide. Cats excreted phenol primarily as phenylsulfate. Pigs excreted the phenol primarily as glucuronide and goat urine contained largely phenylsulfate with some free phenol. Injected phenol was excreted in the spine as free phenol, phenylsulfate and phenylglucuronide. The proportions of each varied with the species and followed the pattern of excretion of the endogenous phenols.

The lack of phenolic oxidation products was related to the rapid conjugation afforded by the intravenous route of administration. Phenylsulfate excretion reached a maximum and remained constant at dosages above 50 mg/kg (saturation). Phenylglucuronide excretion rates progressively increased with increases in dose. The excretion rate of phenylglucuronide in cats was the lowest of all of the animals studied and increased the least with each dose increase. Injected phenylsulfate and phenylglucuronide were rapidly cleared from the plasma and were completly accounted for in the 24-hour urine sample. Similar clinical effects were observed in all species at similar plasma levels. However, cats were more severely affected by given dosages of phenol than were dogs, swine and goats. This was explained by differences in disposition and fate of phenol within the body.

Amphetamine

A similar dose (0.66 mg/kg body weight, calculated as free base) of dl-amphetamine sulphate was administered intravenously to each subject in all of the experiments. The amphetamine concentrations in the biological fluids (blood plasma, cerebrospinal and ocular fluids, urine, bile, gastric fluid and rumen liquor) were determined by a sensitive and specific gas chromatographic method and the concentration of p-hydroxyamphetamine in urine was determined by a colorimetric method.

The disappearance of amphetamine from plasma was a first-order process in all species (Figure 4). Plasma half-lives of 0.62, 1.05, 1.39, 1.40, 2.27, 4.49 and 6.53 hours were found in goats, swine, ponies, rabbits, chickens, dogs and cats respectively, Table VII. The mean biological half-life of this drug in these randomly selected groups of animals varied significantly between species (F-test, $p<0.01$). No sex difference in

TABLE VII.—*Pharmacokinetic Constants Describing the Distribution and Elimination of Amphetamine (1)*

Species (number)	B (ng/ml)	T½ (hr)	β (hr^{-1})	V'd (litre/kg)
Caprine (10)	235	0.62	1.118	3.08
Porcine (9)	330	1.05	0.674	2.23
Equine (5)	281	1.39	0.500	2.61
Lapine (4)	333	1.40	0.498	2.29
Avian (8)	438	2.27	0.305	1.81
Canine (11)	263	4.50	0.159	2.67
Feline (6)	401	6.53	0.108	1.83

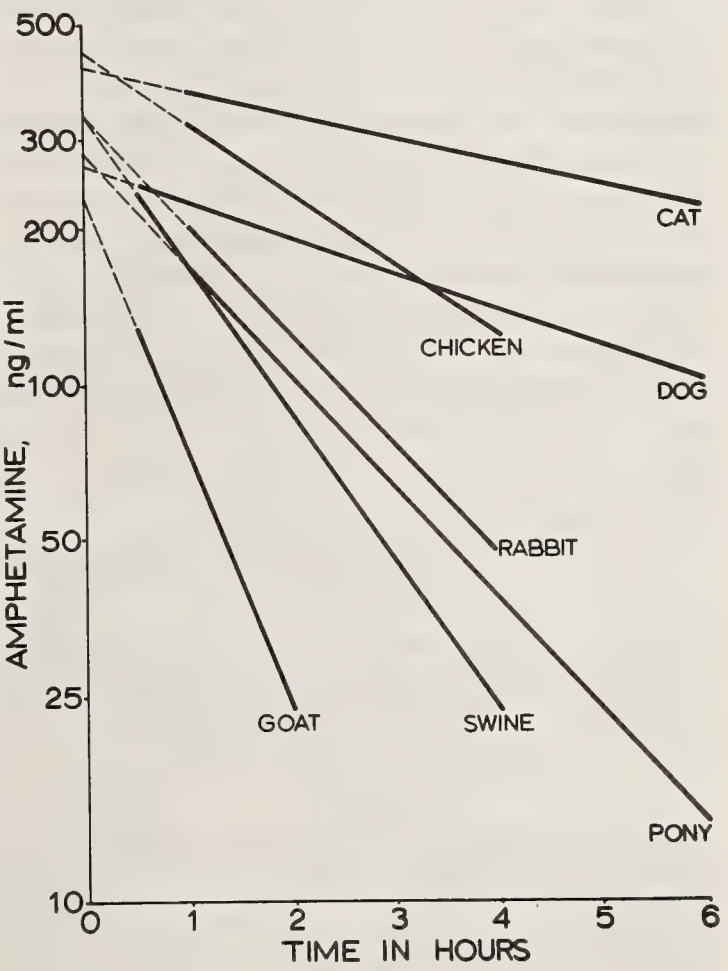


FIGURE 4.—Disappearance of amphetamine from the blood plasma of several species following the intravenous injection of amphetamine sulfate (0.66 mg/kg, calculated as free base).

persistence of the drug was observed in any species studied.

The extent of plasma protein binding of amphetamine was independent of drug concentration over the range of concentrations observed *in vivo* (25–400 ng ml^{-1}). At a drug concentration of 10^{-6} Molar the mean percent binding of amphetamine was 40.7, 39.6, 25.3, 31.0, 14.5, 27.1, 26.4, 40.5, 40.2, 26.0 for goats, swine, ponies, rabbits, chickens, dogs, cats, rats, monkeys and opposum respectively, Table VIII. The extent of plasma protein binding of amphetamine differed significantly between species (F-test, $p<0.01$).

These protein binding values were employed in correcting the apparent specific volumes of distribution of the drug. The corrected volumes of distribution were large in all species, the values obtained for the porcine, equine, lapine and canine species were similar (ca. 3.5 litres/kg). The ruminant animals had a particularly large apparent volume of distribution (5.19 litres/kg) which may have been due to passive diffusion into the rumen of some of the unbound nonionized portion of circulating drug. Cats and chickens, on the other hand, had smaller volumes of distribution for this drug than those estimated for the other animals. The value of k_2 relative to β was directly related to the apparent specific volume of distribution of the drug. The wider the k_2:β ratio the shorter the biological half-life. In goats the value of k_2 was one hundred times that of β while in cats k_2 was fifty times β.

The quantities of unchanged amphetamine, p-hydroxyamphetamine and its glucuronide and sulphate conjugates in cumulative urine samples were measured. A relationship appeared to exist between dietary habit and metabolic pattern of amphetamine. Approximately one-third of the amphetamine injected was excreted unchanged in the urine of carnivorous animals and chickens while a small percentage of the dose was excreted unchanged in the urine of herbivorous animals. The amount of amphetamine which is eliminated by renal excretion, that is, the magnitude of the contribution of $k_{excretion}$ to k_2, may be of importance in determining the effectiveness of urinary pH change upon the biological half-life of this drug. The urinary pH reaction had a profound effect upon the renal clearance and directly influenced the biological half-life of amphetamine in dogs. The renal clearance values provided evidence that amphetamine was probably filtered and then reabsorbed by glomerular filtration and tubular reabsorption. In horses the biological half-life was not significantly influenced (Student's "t" test, $p(<0.05)$ by any urinary pH reaction.

The biliary clearance values and the cumulative amount of unchanged amphetamine in bile (dogs and swine) indicated that this pathway played a minor role in the elimination of this drug from the body.

TABLE VIII.—*Total Plasma Protein Concentration and Extent of Protein Binding of Amphetamine in Various Species of Animals*

Species	Amphetamine concentration: 10^{-6} Molar			
	% bound amphetamine		Plasma protein conc. (g%)	
	Mean	S.E.M.	Mean	S.E.M.
Equine (9)	25.3	2.6	7.5	0.3
Caprine (12)	40.7	1.7	6.6	0.3
Porcine (6)	39.6	3.0	6.4	0.6
Canine (17)	27.1	1.5	6.7	0.3
Feline (12)	26.4	1.2	7.3	0.2
Lapine (4)	31.0	2.9	5.5	0.3
Avian (6)	14.5	0.9	3.0	0.3
Monkey (11)	40.2	0.7	7.8	0.2
Opossum (4)	26.0	4.0	6.8	0.4
Rat (4)	40.5	3.0	6.1	0.8

Quinidine

The disappearance of quinidine from the blood plasma of the various species is shown in Figure 5. First-order kinetics were followed in all species. The most remarkable feature of these data, aside from differences in slopes of the various curves, is the high plasma concentrations of the drug in swine.

Values for kinetic constants are tabulated in Table IX. The plasma half-life was shortest in goats and longest in dogs and swine. On extrapolating to zero time, the mean values for C_0 (drug concentration at zero time) varied from 2.68 mg/L in goats 9.69 mg/L in swine. Values obtained in ponies were not comparable because the dose of quinidine administered was half that given to the other species. Values for V'd (apparent specific volume of distribution) varied among the different species and were indica-

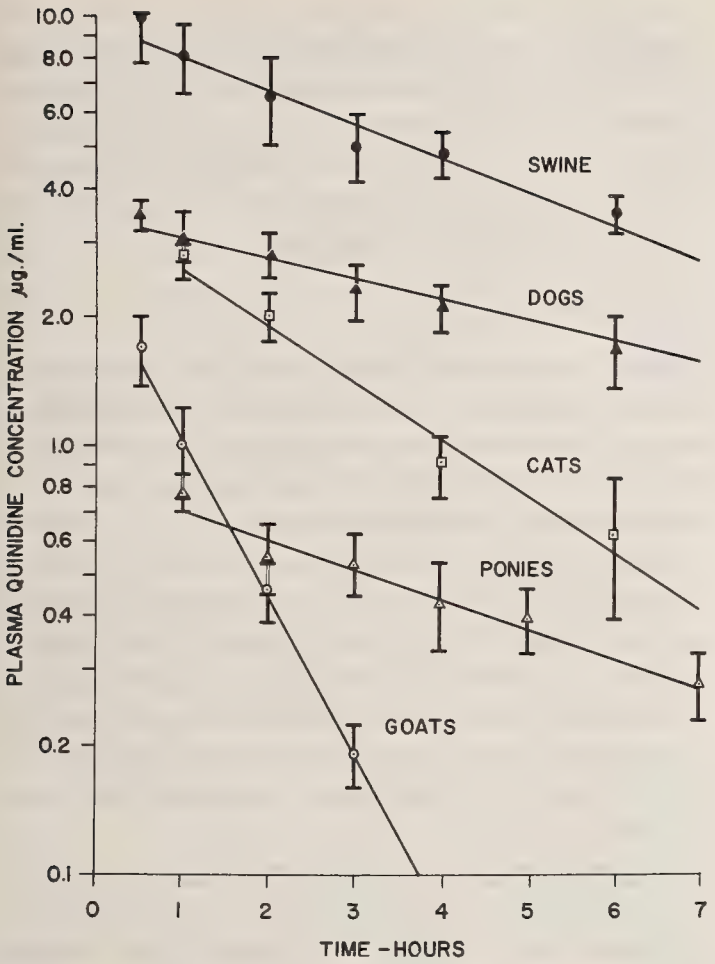


FIGURE 5.—Disappearance of quinidine from the blood plasma of several species following the intravenous injection of quinidine hydrochloride (10 mg/kg, calculated as free base). Ponies were given a dose of 5 mg/kg. Each point represents the mean drug concentration and each vertical bar equals ± 1 S.E.M.

tive of extensive tissue distribution and sequestration of the drug. The corrected V'd values are of theoretical interest in that they represent the apparent specific volumes of distribution for the respective species if all of the drug in the blood plasma were freely diffusible, i.e., not bound to plasma protein.

Results of the study of plasma protein binding are summarized in Table X. The extent of quinidine-protein binding varied inversely with drug concentration in all species. Species differences in the extent of protein binding of drug were observed. Quinidine was bound least in caprine plasma (77%) and most in porcine plasma (98%) at a drug concentration of 5µg/ml. Values for the extent of binding in the plasma of other species studied were intermediate between these two extremes.

The pattern of species differences in the kinetic constants for quinidine differed somewhat from patterns for other bases such as amphetamine, pentazocine, and chloramphenicol. The half-lives of quinidine were similar in swine and dogs (about 5.5 hrs.), somewhat shorter in ponies (4.4 hrs.) and much shorter in cats (1.9 hrs.) and goats (0.85 hrs.). The explanation for these species differences in rate of disappearance of quinidine is obscure at the present time but probably is a manifestation of differing rates of biotransformation. The principal pathway for biotransformation of quinidine is hy-

TABLE IX.—*Kinetic Constants for the Disappearance of Quinidine from the Blood Plasma of Domesticated Animals*

Species	Co (mg./L.)	T ½ (hr.)	kd ($hr.^{-1}$)	V'd (L./kg.)	Corrected V'd (L./kg.)
Ponies	0.83 ± 0.11	4.37 ± 0.36	0.16 ± 0.015	6.32 ± 0.71	34.0
Goats	2.68 ± 0.73	0.85 ± 0.08	0.82 ± 0.080	4.89 ± 0.94	16.3
Swine	9.69 ± 1.88	5.42 ± 1.56	0.13 ± 0.031	1.25 ± 0.29	49.5
Dogs	3.56 ± 0.33	5.59 ± 0.83	0.14 ± 0.016	2.91 ± 0.23	22.0
Cats	4.65 ± 0.41	1.87 ± 0.28	0.40 ± 0.056	2.22 ± 0.20	21.5

Co = plasma concentration of drug at zero time. T ½ = plasma half-life. kd = apparent first-order disappearance rate constant. V'd = apparent specific volume of distribution of the drug. Corrected V'd is the apparent specific volume of distribution of free drug after correction for plasma protein binding. Values are reported as the mean ± S.E.M.

TABLE X.—*Effect of Drug Concentration on the Extent of Quinidine-Plasma Protein Binding In Vitro*

Species	Quinidine Concentration (µg/ml.)				Protein Concentration (g./100 ml.)
	5	10	25	50	
Ponies	82 ± 0.8	69 ± 3.7	63 ± 5.7	56 ± 3.5	6.88 ± 0.61
Goats	77 ± 6.9	72 ± 7.7	67 ± 7.5	67 ± 5.3	5.98 ± 0.58
Swine	98 ± 2.1	85 ± 2.5	70 ± 6.6	61 ± 5.2	6.48 ± 0.85
Dogs	87 ± 5.4	75 ± 1.4	72 ± 2.9	66 ± 3.5	5.53 ± 0.58
Cats	90 ± 5.8	85 ± 9.3	69 ± 7.5	58 ± 4.4	7.63 ± 0.48
Human beings	89 ± 7.9	82 ± 7.3	71 ± 7.9	69 ± 6.7	6.83 ± 0.10

Values for the extent of binding of drug are expressed as the mean percent bound ± S.E.M.

droxylation of the quinuclidine nucleus while hydroxylation of the quinoline ring is a minor, secondary pathway.

Quinidine was unusual in our experience in that the cat was able to eliminate it more rapidly than were swine, dogs, and ponies. The cat eliminated amphetamine, pentazocine, salicylate and oxyphenbutazone much more slowly than the other species. Presumably, quinidine was different because conjugation mechanisms were not involved. The rapid rate of elimination of quinidine from the plasma of goats was believed to be due to rapid diffusion and subsequent trapping of quinidine in the rumen. The data obtained from ponies are not directly comparable to those for other species because it was necessary to employ a smaller dosage (5 mg/kg) of the drug.

Quinidine rapidly diffused from the blood and concentrated in tissues. Distribution equilibrium was complete within 30 minutes. Thus the kinetics of quinidine could be described adequately by a one-compartment open model in all species. If we employ the values for C_0 and assume a blood volume equivalent to 7% of the body weight, only 1.2, 1.8, 2.5, 3.3 and 6.8% of the dose was in the blood of ponies, goats, dogs, cats, and swine, respectively after diffusion equilibrium was attained. The sequestration of quinidine by tissues was further corroborated by the large values obtained for apparent specific volumes of distribution. These varied from 1.25 L/kg in swine to 6.32 L/kg in ponies. The differences in apparent volumes of distribution and percents of dose in the blood were related to the extents of quinidine-plasma protein binding among the species. Quinidine was 98% bound in swine (smallest V'd) and 82% bound in ponies (largest V'd). If the drug were extensively bound to plasma proteins there would be less free drug available for diffusion. Values for C_0 were inversely related to extent of protein binding so the differences in plasma concentrations among species may be explained on the basis of protein binding.

The nature of the interaction of quinidine with plasma proteins is different from that observed with amphetamine and chloramphenicol because extent of binding of the latter drugs was independent of drug concentration. The fact that the extent of binding of quinidine was inversely related to drug concentration suggests the presence of specific binding sites in the plasma proteins. Since diffusion equilibrium occurred rapidly with high tissue/plasma concentration ratios we would expect the proteins to have a low affinity for the drug.

Pentazocine

Pharmacokinetic parameters and pharmacologic effects were studied in ponies, goats, swine, dogs and cats. A fluorometric procedure was developed for the assay of pentazocine were observed among the species studied.

Cats. Mydriasis and sialosis were constant findings. Five out of six cats were mildly depressed from 30 minutes through four hours. One cat became aggressive and excited for a period of five hours. The subjects did not seem to resent the venipunctures during the period after injection of the drug. Recovery was uneventful.

Dogs. Profuse salivation, diarrhea, mydriasis and pollakiuria were prominent signs during the first hour. Slight depression and ataxia lasted for 1½–2 hours.

Swine. Sialosis and emesis occurred in all swine from 1 to 2 hours after administration. Pollakiuria was observed in three animals during the first hour. Polypnea was a constant finding from 1 to 3 hours; this was not due to ambient temperature as other swine in the barn were unaffected. Swine were depressed from the first through third hours. The animal caretaker was of the opinion that the swine were more difficult to catch and handle when under the influence of the drug.

Ponies. Polypnea occurred in all subjects from 30 minutes to 1 hour and unsteadiness of gait was observed from 30 minutes to 3 hours after administration. Fetuses in two mares showed increased activity from 15 minutes to 75 minutes after injection. Both mares delivered normal foals three weeks after the experiment.

Goats. No behavioral or autonomic effects were seen.

No other significant untoward effects were observed in any species. No attempt was made

to investigate analgesic efficacy although the subjects seemed to tolerate the blood collection procedures better after the absorption of the drug.

Pharmacokinetics. The modes of absorption and disappearance of pentazocine from the blood plasma of the various species were studied. Peak plasma levels of pentazocine occurred at 15 minutes in dogs, goats and swine; at 30 minutes in ponies and at 1 hour in cats. Disappearance of the drug from plasma followed first-order kinetics in all species except dogs. A biphasic mode of disappearance was noted in canine subjects. A rapid fall in pentazocine concentration ($T\frac{1}{2}$ − 22 minutes) occurred between 15 minutes and 1 hour. This was followed by a slower ($T\frac{1}{2}$ − 150 minutes) rate of decay from 1 hour through 7 hours.

Values for kinetic constants are tabulated in Table XI. The plasma half-life was shortest in dogs and longest in ponies. On extrapolating to zero time, the mean values for Co (drug concentration at zero time) varied from 0.52 mg/L in goats to 1.08 mg/L in cats. Values for V'd (apparent specific volume of distribution) were indicative of extensive tissue distribution and sequestration of the drug.

Several effects observed following the administration of pentazocine were similar to certain actions of morphine. Pollakiuria, mydriasis, sialosis and emesis are common to both drugs. The mechanism of the polypnea in swine and ponies is uncertain but morphine-induced panting has been attributed to an alteration in the equilibrium point of the hypothalamic heat-regulatory mechanism. The reason for the lack of overt drug effects in the goats is obscure but I have had similar experience with morphine in this species.

In general, the duration of the depressant action of pentazocine in the various species coincided well with the persistence of plasma levels. However, depression persisted for a longer time in dogs and a shorter time in ponies than one might anticipate on the basis of disappearance of the drug from the plasma. A period of depression longer than the presence of plasma levels of pentazocine could be explained by the observation in cats that brain concentrations of the drug were slightly higher than blood concentrations.

The plasma half-life of pentazocine was shorter than that reported in man for all of the species studied. These differences are most likely related to species differences in rates of metabolism. The half-life of pentazocine in cats was intermediate between that of morphine (3.05 hours) and meperidine (42 minutes). Similar comparisons cannot be made in the other species because of lack of information.

Pentazocine rapidly left the blood and concentrated in tissues. This is typical of the body disposition of basic amines. At peak plasma levels, assuming a blood volume of 7% of the body weight, only 1.7, 1.0, 1.0, 1.0 and 1.2% of the dose was present in the blood of cats, dogs, swine, goats and ponies, respectively. The sequestration of the drug in the body tissues is further supported by a consideration of the apparent specific volumes of distribution. These varied from 2.78 L/kg in the cats to 5.77 L/kg in the goats. The higher values found for the herbivorous animals could be related to diffusion and concentration of the drug in the rumen of the goat or large colon of the pony. Ferrari[13] found that pentazocine concentrated in muscle, liver and the gastrointestinal tract of cats.

The dose of pentazocine employed in this investigation (3 mg/kg) was higher than that studied in human patients (0.6 mg/kg). The dose employed was well below the amount reported to produce toxicity in animals. As expected, the peak blood levels obtained in all species, except cats, were nearly five times the concentrations observed by Berkowitz et al.[14] in their human subjects. The higher plasma drug concentration observed in the cats was attributed to a smaller specific volume of distribution.

Chloramphenicol

Chloramphenicol was studied in ponies, goats, swine, dogs and cats following intravenous, intramuscular and oral administration (10 mg/lb). The disappearance of chloramphenicol from the blood plasma of the various species following IV administration is shown in Figure 6. First-order kinetics were followed in

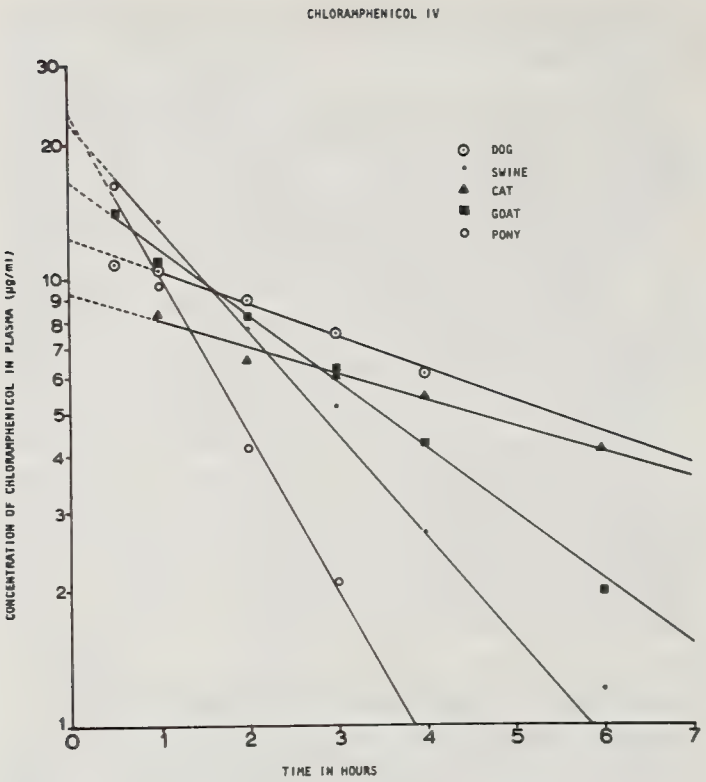


FIGURE 6.—Disappearance of chloramphenicol from the plasma of several species following the intravenous injection of chloramphenicol (22 mg/kg). Each point represents the mean value from eight animals.

all species and distribution equilibrium was attained rapidly (less than 30 min.).

Values for the kinetic constants are given in Table XII. The half-life of the drug in the plasma was longest in cats and shortest in ponies. Values for V'd varied among species and were large (in excess of total body mass). This indicates that chloramphenicol, like the other bases studied, is sequestered in the tissues. This drug was unusual in that the cat had the largest specific volume of distribution while that in the ruminant was much less.

TABLE XI.—*Kinetic Constants for the Disappearance of Pentazocine from the Blood Plasma of Various Species of Domestic Animals*

Species	Co mg./L.	T½ (min.)	k'd hr^{-1}	V'd L./kg.
Ponies	0.59	97.1	0.0071	5.09
Goats	0.52	51.0	0.0136	5.77
Swine	0.63	48.6	0.0143	4.76
Dogs	0.85	22.1	0.0313	3.66
Cats	1.08	83.6	0.0083	2.78

Co is the value for plasma concentration of drug at zero time.
T½ is the plasma half-life.
k'd is the apparent first-order disappearance rate constant.
V'd is the apparent specific volume of distribution of the drug.

TABLE XII.—*Kinetic Constants for the Disappearance of Chloramphenicol from the Blood Plasma of Domesticated Animals*

Species	Co (mg/L)	T½ (hrs)	Kd (hr^{-1})	V'd L/kg	V'd* L/kg.
Dogs	12.4	4.2	0.24	1.77	2.96
Cats	9.3	5.1	0.14	2.36	3.69
Swine	21.0	1.3	0.53	1.05	1.61
Goats	16.5	2.0	0.35	1.33	1.91
Ponies	21.5	0.9	0.77	1.02	1.46

Co = plasma concentration of drug at zero time. T½ = plasma half-life. Kd = apparent first-order disappearance rate constant. V'd = apparent specific volume of distribution of the drug. Values are reported as the mean ± S.E.M. V'd* = Apparent specific volume of distribution corrected for protein binding.

Results of the study of extent of chloramphenicol-plasma protein interaction are summarized in Table XIII. All determinations were made at an initial drug concentration of 20 µg/ml. Values varied from 30.1% in the goat to 46% in human plasma. The variation observed among the various species probably is due to species differences in albumin because there was no relationship between extent of binding and protein concentration. The effect of drug concentration on the extent of binding of chloramphenicol was studied in feline, canine, equine, caprine, porcine and human plasma at drug concentrations of 10, 20, 40, 60 and 100 µg/ml. Within this range the extent of binding was independent of drug concentration. Accordingly, we could correct the values calculated for V'd to obtain the V'd for free drug. These values for the various species are listed in Table XII.

Chloramphenicol in gelatine capsules was given orally (10 mg/lb) to each animal. The time course of drug concentration in plasma are shown in Figure 7. Peak levels occurred at two hours in swine and dogs and at three hours in ponies and cats. Detectable concentrations of chloramphenicol in the blood of goats were not

TABLE XIII.—*Extent of Chloramphenicol-Plasma Protein Binding*

Species	Drug Bound to Plasma Protein (%)	Plasma Protein Concentration g/100 ml
Dog	39.5 ± 3.4	8.1 ± 0.16
Cat	37.7 ± 2.1	7.9 ± 0.25
Swine	35.1 ± 2.5	6.0 ± 0.16
Goat	30.1 ± 2.6	7.8 ± 0.34
Pony	30.4 ± 2.0	8.7 ± 0.19
Human	46.0 ± 0.46	8.1 ± 0.24

Values are expressed as the means ± 1 S.E.M.

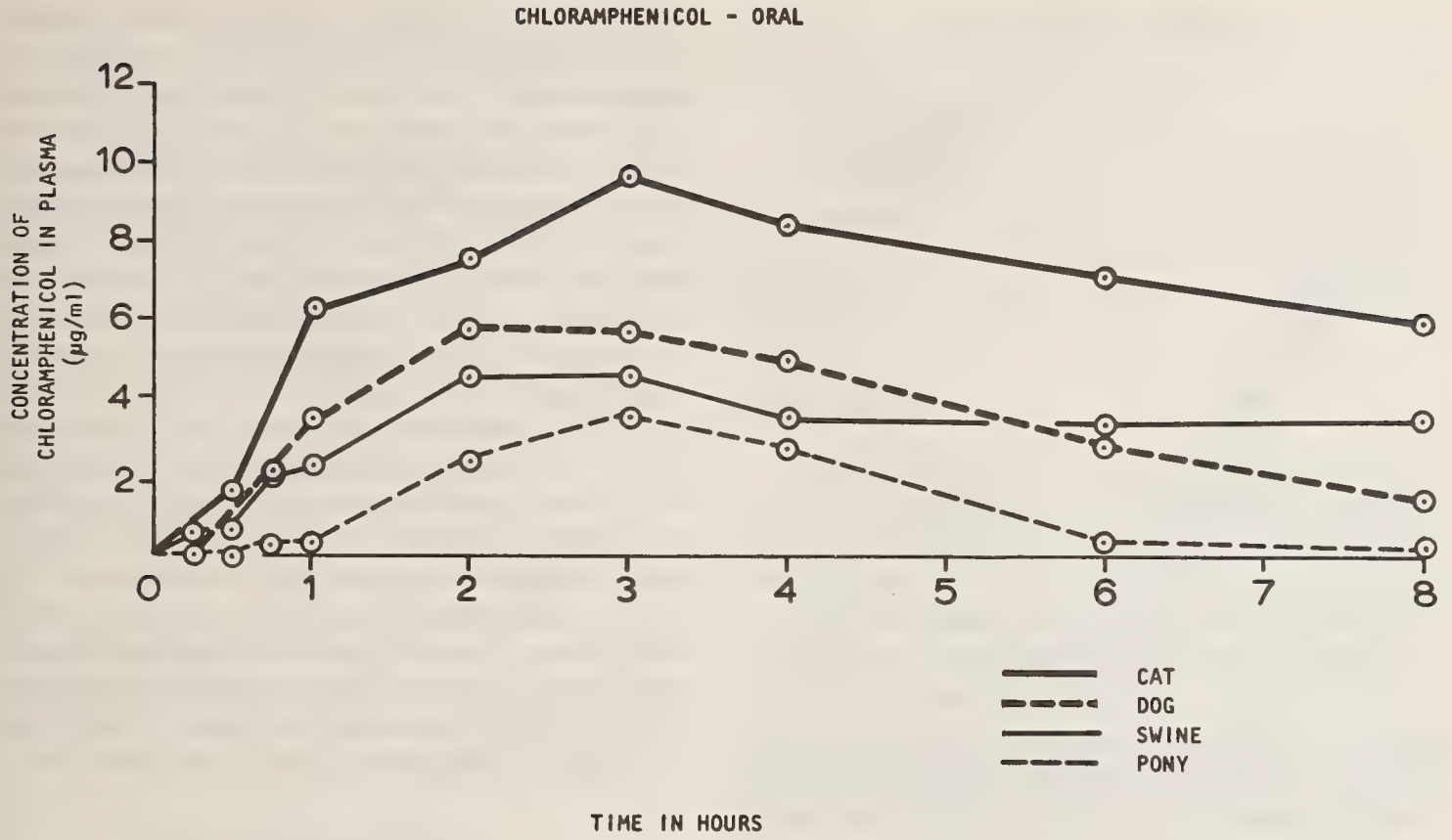


FIGURE 7.—Concentrations of chloramphenicol in plasma following the oral administration of chloramphenicol (22 mg/kg). Each point represents the mean value from eight animals. Chloramphenicol was not detectable in the plasma of goats following oral administration.

found after oral administration. This substantiates the finding of Theodorides et al.[15] that chloramphenicol is reduced to aryl amines by the ruminal microflora.

Figure 8 illustrates similar information following intramuscular injection of chloramphenicol (10 mg/lb). Peak plasma concentrations were obtained by one hour or less in all species.

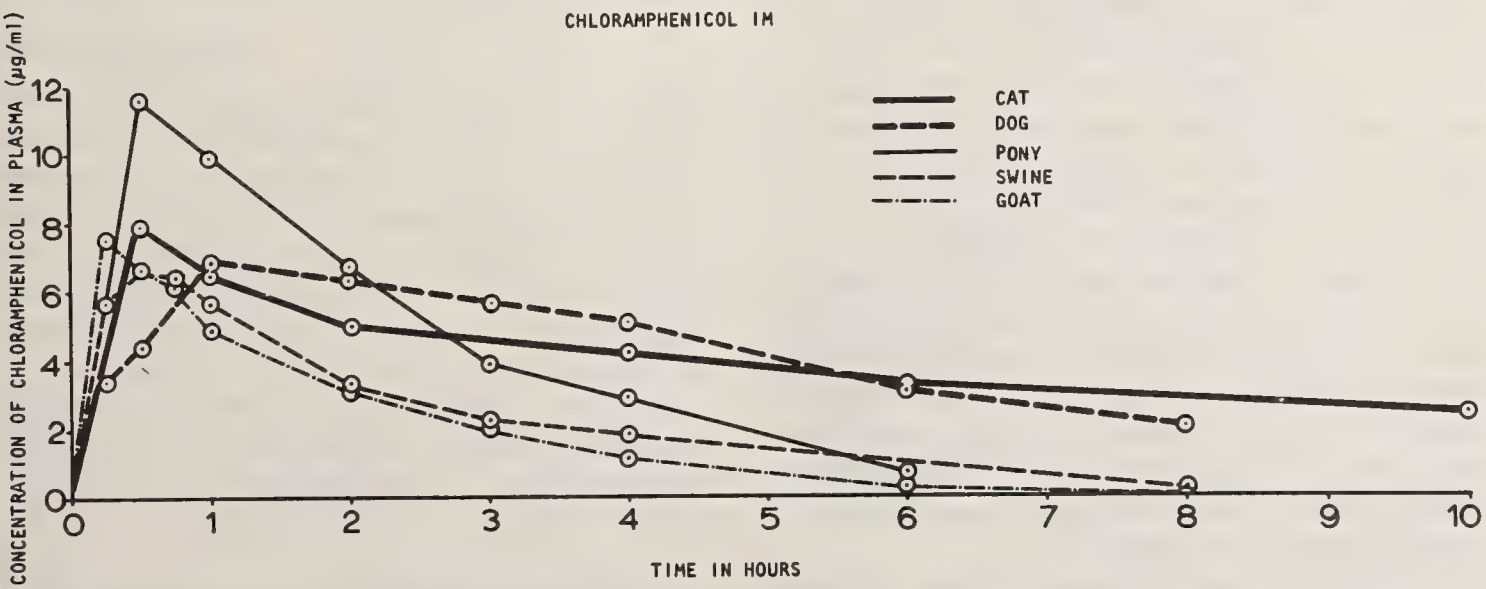


FIGURE 8.—Concentrations of chloramphenicol in plasma following the intramuscular injection of chloramphenicol (22 mg/kg.). Each point represents the mean value from eight animals.

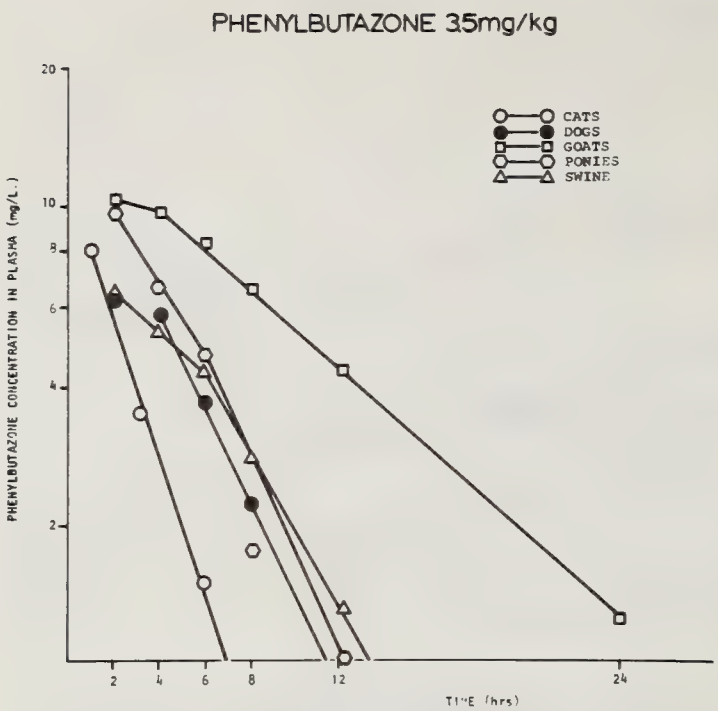


FIGURE 9.—Disappearance of phenylbutazone from the plasma of several species following intravenous injection (3.5 mg/kg).

The drug appeared to be readily absorbed from the depot site in all species.

Phenylbutazone

Phenylbutazone was studied in dogs, cats, swine, ponies and goats following I.V. administration (at three dosage levels—3.5, 10 and 25 mg/kg) and following oral administration of 10 mg/kg. The concentration-time relationships are shown in Figures 9–12. The time course of this drug in the plasma of the various species was unusual in that carnivores eliminated it more rapidly than did the other species. Phenylbutazone had a longer half-life in all of the species than any other drug studied. The specific volume of distribution were somewhat different in that the drug had a smaller apparent volume of distribution in the ruminant animal than in swine or ponies. Blood concentrations increased proportionately by increasing the dose from 3.5 to 10 mg/kg but a further increase from 10 to 25 mg/kg did not result in proportionate blood levels.

These effects are accounted for on the basis of plasma protein binding of the drug. At low drug concentrations 10–20 mg/L the drug was nearly 100% bound in plasma from goats, swine and humans, 70% in ponies and 85% in dogs and cats. The extent of binding decreased with increasing drug concentration except in ponies. Drug concentration had no effect on extent of binding in equine plasma throughout the range of 10 to 100 mg/L. The species differences observed in the absorption of phenylbutazone following oral administration are difficult to explain. Highest peak blood levels occurred in goats and the lowest were attained in swine. Absorption was relatively slow in all species.

The principal metabolites isolated from the urine were oxyphenbutazone, the glucuronide and ethereal sulfate of oxyphenbutazone. Species differences were observed in distribution of these compounds in 24-hour urine samples. Oxyphenbutazone was the principal metabolite isolated from the urine of ponies, swine, and cats. The glucuronide accounted for most of the drug eliminated in the urine of dogs and goats. Sul-

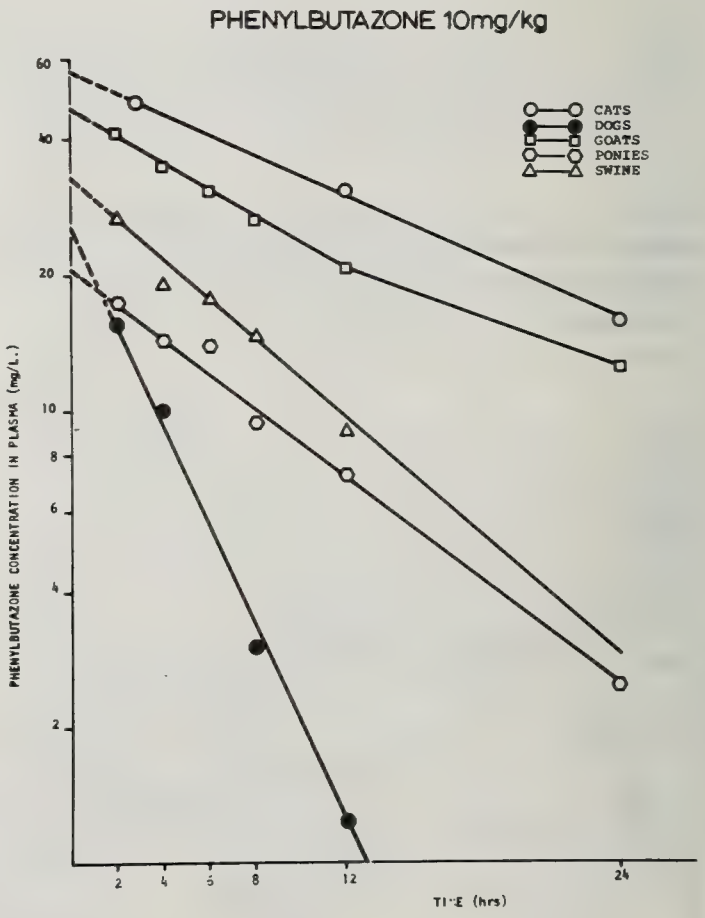


FIGURE 10.—Disappearance of phenylbutazone from the plasma of several species following intravenous injection (10 mg/kg).

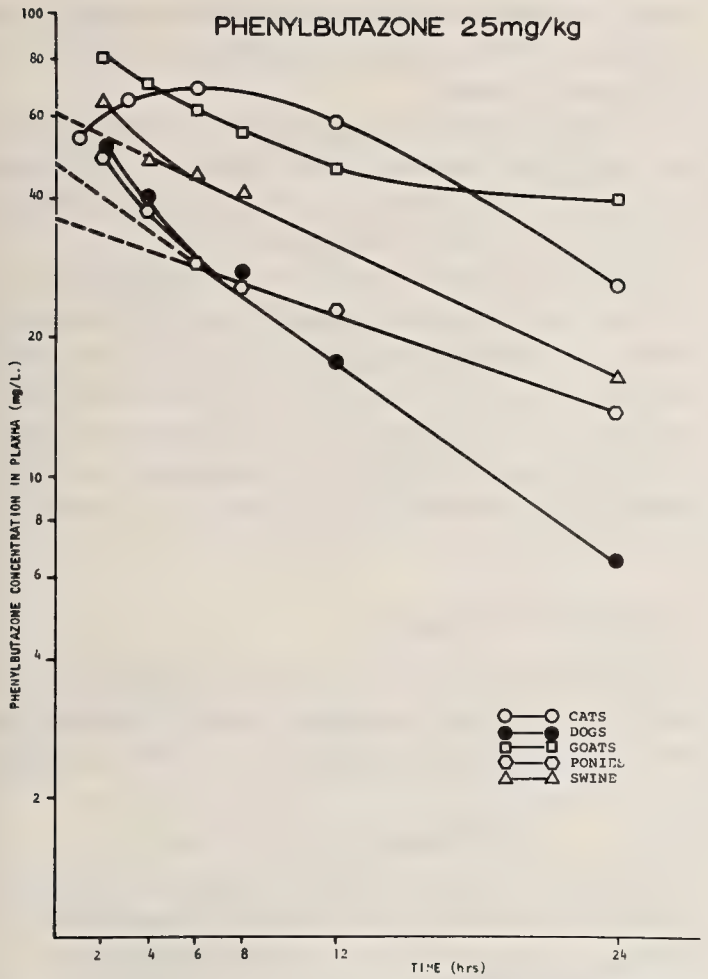


FIGURE 11.—Disappearance of phenylbutazone from the plasma of several species following intravenous injection (25 mg/kg).

fate was a minor product in all species but was quantitatively important in ponies and goats.

Oxyphenbutazone

Oxyphenbutazone was studied in the same manner as phenylbutazone. These data are shown in Figures 13–16. This drug was eliminated more rapidly in all species, except the cat, than was phenylbutazone. These data further substantiate the importance of biotransformation in species differences in the pharmacokinetics of drugs. Phenylbutazone was rapidly oxidized to oxyphenbutazone in the cat and hence had a short half-life. Oxyphenbutazone was quite persistent in the plasma of cats because of the relative deficiency of glucuronyl transferase.

The extent of oxyphenbutazone-plasma protein binding in all species was appreciably lower than that observed in human plasma. This was not as important factor in the pharmacokinetics of oxyphenbutazone as it was in the case of phenylbutazone.

Following oral administration, oxphenbutazone was absorbed to about the same extent and at the same rate as phenylbutazone in cats and ponies. It was better absorbed by swine and less well absorbed in goats and dogs than was phenylbutazone.

Pentobarbital

Pentobarbital was studied in 16 ponies, 16 swine, 8 dogs and 8 cats. The kinetic parameters were evaluated in all animals following I.V. administration of the drug at a dosage of 10 mg/kg. Subsequently, each species group was divided into four treatment groups. The control group received no treatment, other groups received 1, 2.7 or 7.3 mg/kg of pentobarbital orally, each day, for six months. The kinetic parameters were evaluated in each individual (10 mg/kg, I.V.) at one-month intervals. In addition, studies were conducted in kids, pup-

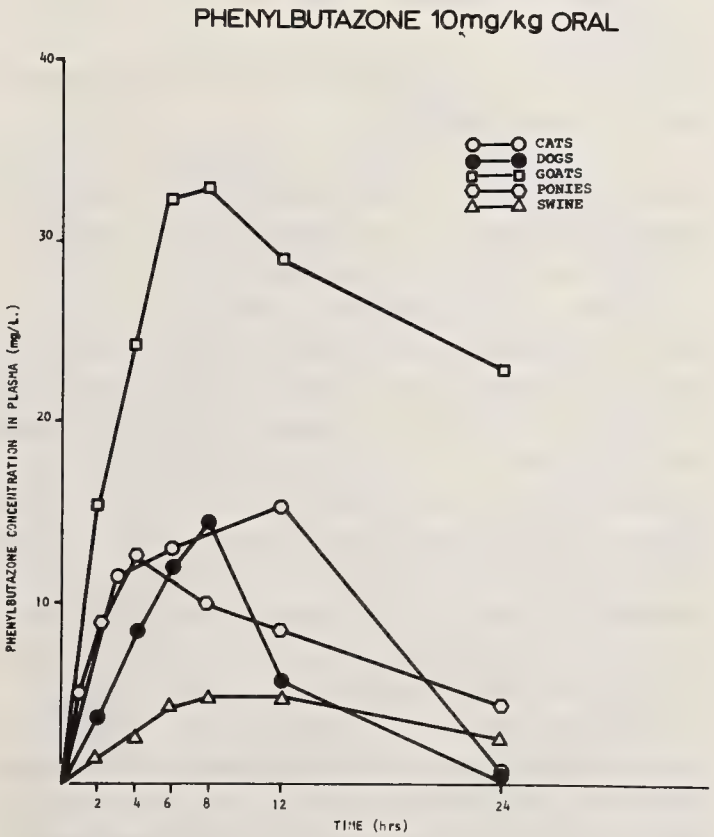


FIGURE 12.—Concentrations of phenylbutazone in the plasma following oral administration (10 mg/kg).

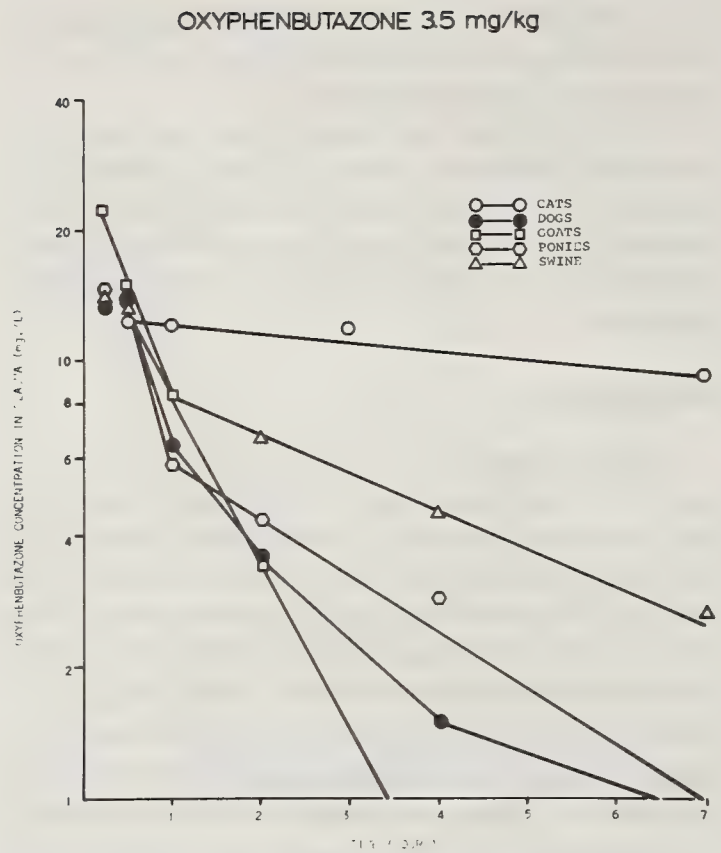


FIGURE 13.—Disappearance of oxyphenbutazone from the plasma of several species following intravenous injection (3.5 mg/kg).

pies, pigs and foals born of dams which were receiving the drug.

Figure 17 shows the disappearance of drug from the plasma of goats and dogs following an anesthetic dose of pentobarbital. The drug was given to effect, blood samples were collected periodically, and the subjects reflexes were evaluated periodically. These data indicated that the species difference in duration of action of drug is related to kinetic considerations rather than to differences in receptor sensitivity. Reflexes reappeared at almost identical drug concentrations in blood. The difference in duration of action was due to the influence of distribution volumes on the overall kinetics. Disappearance of drug from the plasma of cats were very similar to that observed in the case of dogs.

Disappearance of pentobarbital from the plasma of various species following I.V. administration of drug (10 mg/kg) is shown in Figure 18. The specific volumes of distribution are nearly identical for ponies, swine and dogs. That for the goat is larger, although it isn't apparent from the figure because the sampling period wasn't long enough to adequately define the elimination phase.

Plasma protein binding didn't have a significant influence on the overall kinetics as species differences were not observed. Extent of binding was about 70% in all species.

Chronic administration of pentobarbital to the various species did not produce a consistent effect on kinetic parameters during the first three months. At the fourth, fifth and sixth months, the half-life of the drug was increased by about 100%. Induction of enzymes, as manifested by a shorter half-life was not observed in any subject.

Tetraethylammonium

The kinetics of disappearance of TEA was investigated in dogs, cats and goats following intravenous administration of TEA (50 mg and 2

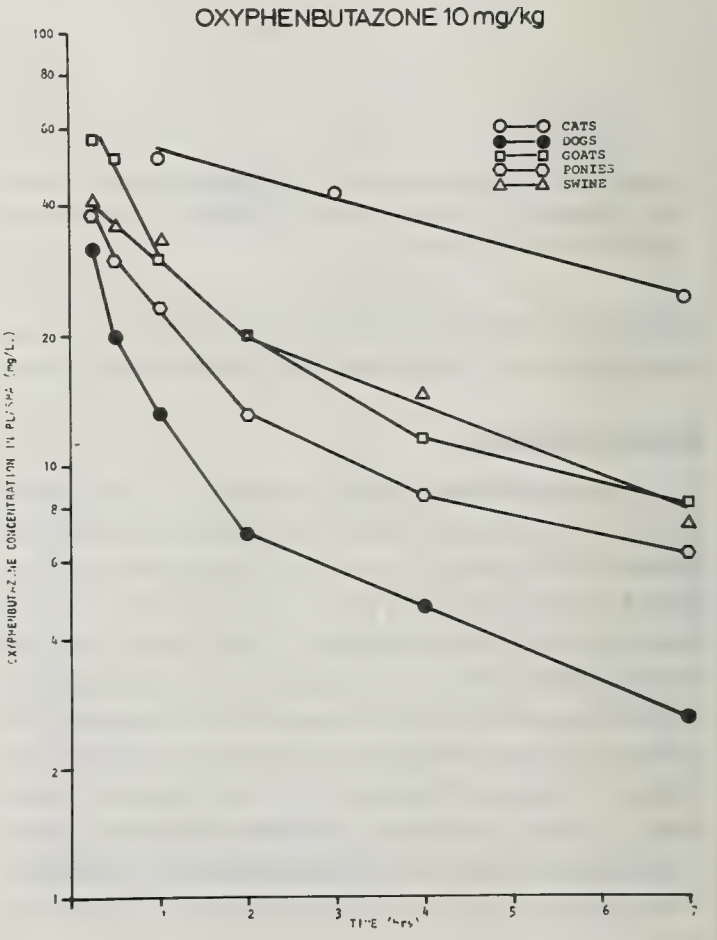


FIGURE 14.—Disappearance of oxyphenbutazone from the plasma of several species following intravenous injection (10 mg/kg).

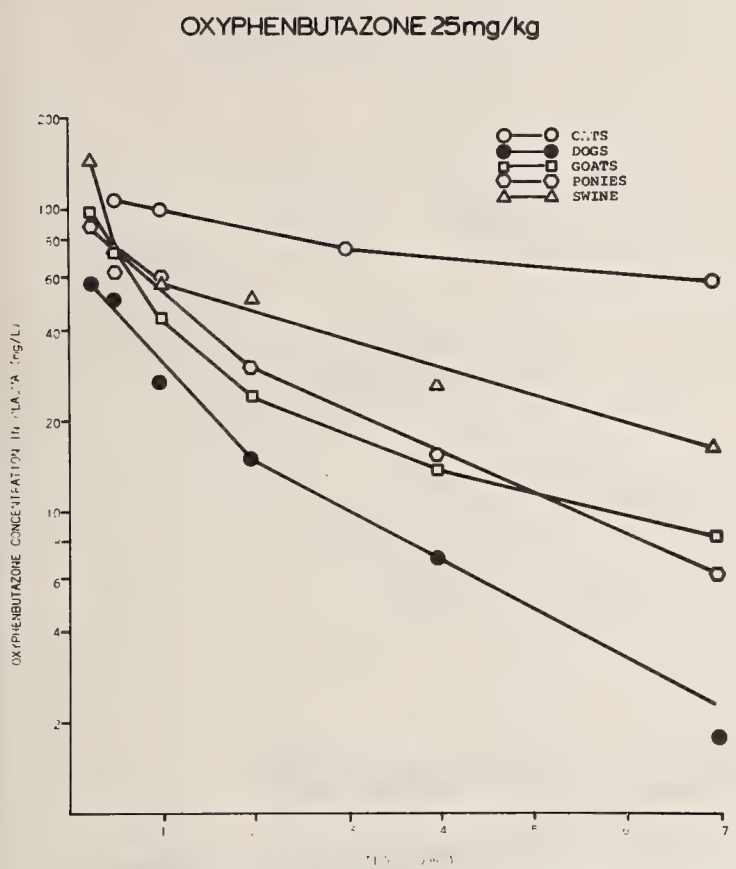


FIGURE 15.—Disappearance of oxyphenbutazone from the plasma of several species following intravenous injection (25 mg/kg).

μCi/Kg). This drug was of interest to us because it is not metabolized, is a strong base which is completely ionized at physiologic pH and is highly polar. Dogs, cats and goats were chosen because for most of the drugs studied previously, these species represented extremes for the kinetic constants, i.e., most drugs were most slowly eliminated from cats, most rapidly eliminated from goats while the dogs were intermediate.

Elimination of this drug was first-order in all three species. The time-concentration profile for TEA in all three species is illustrated in Figure 19. It may be observed that it required longer for distribution equilibrium to be attained in the goat than in the dog.

The kinetic constants for TEA are tabulated in Table XIV. Differences in the rate constants (Kd and T½) were not significant among species. Values for Co and V'd were not significant between the dog and cat. These values in the dog were significantly different ($P < 0.05$) from the goat and in the cat they were highly different ($P < 0.001$ and $P < 0.005$) from the goat.

TABLE XIV.—*Kinetic Constants for the Disappearance of Tetraethylammonium from the Blood Plasma*

Species	Co (mg/L)	T½ (hr)	Kd (hr^{-1})	V'd (L/kg)
Cats	9.9 ± 0.60	0.76 ± 0.06	0.94 ± 0.05	1.0 ± 0.08
Dogs	6.9 ± 1.50	0.94 ± 0.10	0.76 ± 0.08	1.6 ± 0.26
Goats	2.8 ± 0.38	0.99 ± 0.09	0.73 ± 0.06	4.0 ± 0.74

Co = plasma concentration of drug at zero time. T½ = plasma half-life. Kd = apparent first-order disappearance rate constant. V'd = apparent specific volume of distribution of the drug. Values are reported as the mean ± S.E.M.

This study would confirm that species differences in rate of elimination of drugs are due largely to differences in rates of biotransformation rather than in differences in excretory mechanisms. Distribution appears to have little influence on the overall elimination rate because the goat had a four-fold greater V'd than the cat, yet the T½ values were essentially the same.

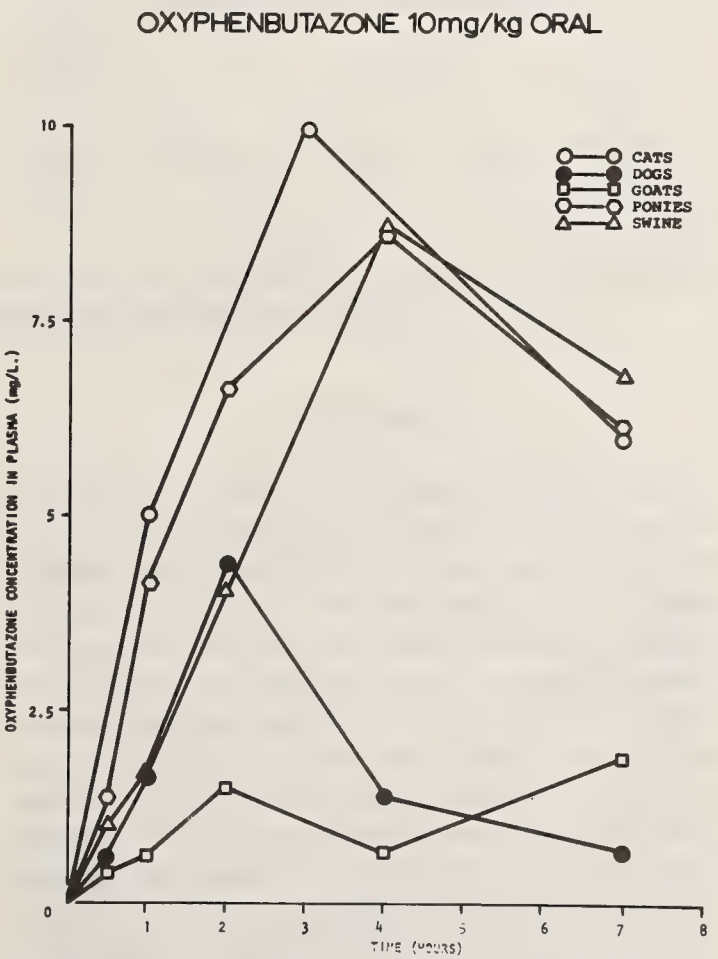


FIGURE 16.—Concentrations of oxyphenbutazone in the plasma following oral administration (10 mg/kg).

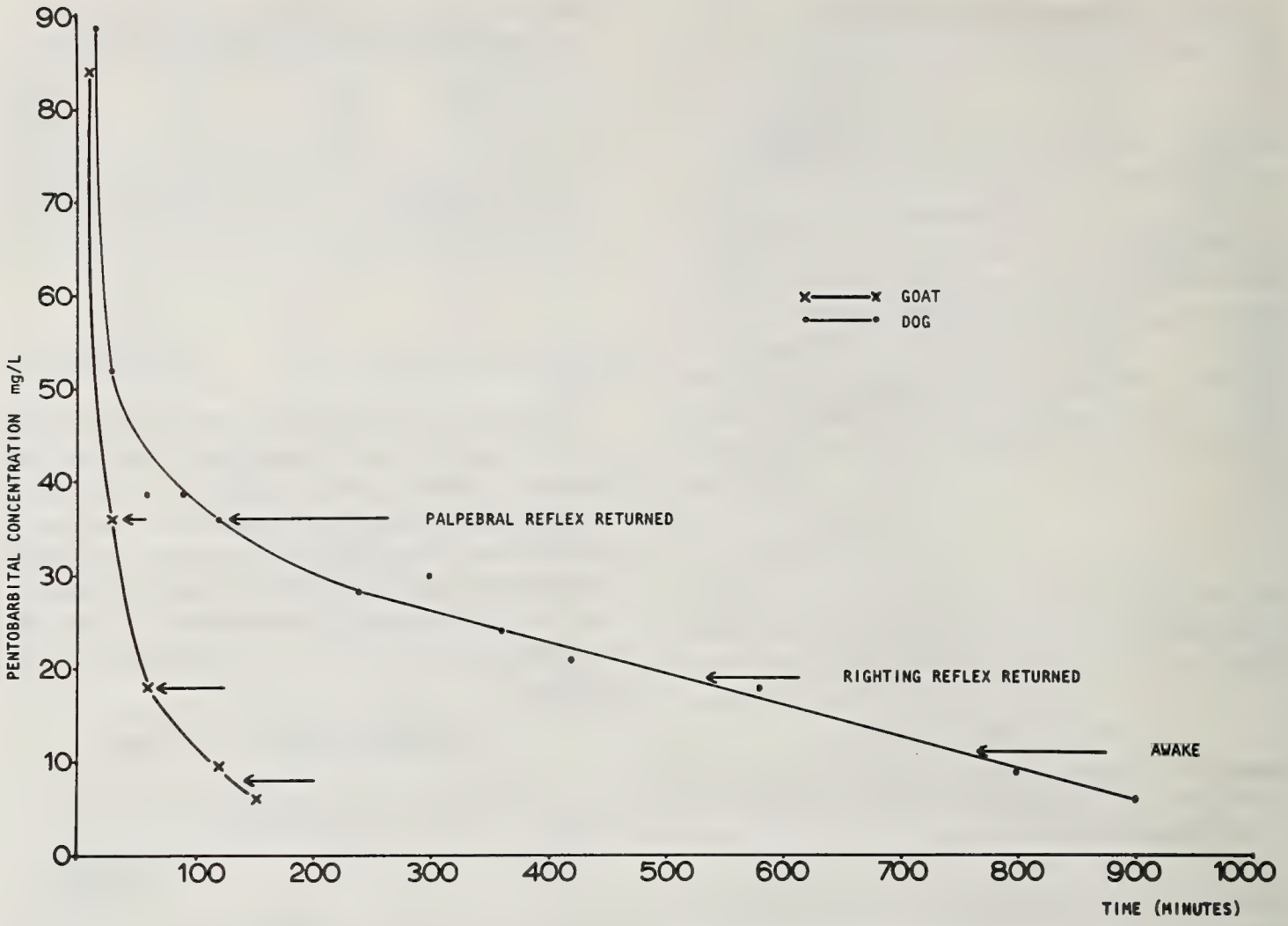


FIGURE 17.—Concentrations of pentobarbital in plasma of dogs and goats following intravenous administration (25 mg/kg). Arrows indicate the drug concentrations and times at which reflexes returned.

SUMMARY

The major objectives of this investigation were the definition of species differences in pharmacokinetics of various drugs, the identification of urinary metabolites and effects of chronic administration on these parameters. These objectives were attained. The scope of the problem of species differences was well delineated by the results reported here. With several drugs the entire spectrum of possible disappearance rates was represented by the species studied. For example, salicylate and phenol were very rapidly eliminated from the plasma of goats and ponies but were slowly eliminated from cats. On the other hand, drugs which were metabolized by oxidative mechanisms were more rapidly eliminated by the cat. There were few general principles to be derived during the course of these studies. Sex differences did not occur with most drugs and are not considered to be an important phenomenon.

It is apparent from these studies, that in most cases, the crucial factor in determining species differences in persistence of drugs is that of rate of biotransformation. Species differences in elimination of drugs which are transformed primarily by conjugation mechanisms are more nearly predictable than are compounds which are metabolized by oxidation or reduction. Herbivorous animals had the greatest capacity for conjugation of xenobiotics, carnivores had the least and omnivores were intermediate. It appears in some cases, that animals which were handicapped in their ability to conjugate drugs were well-endowed

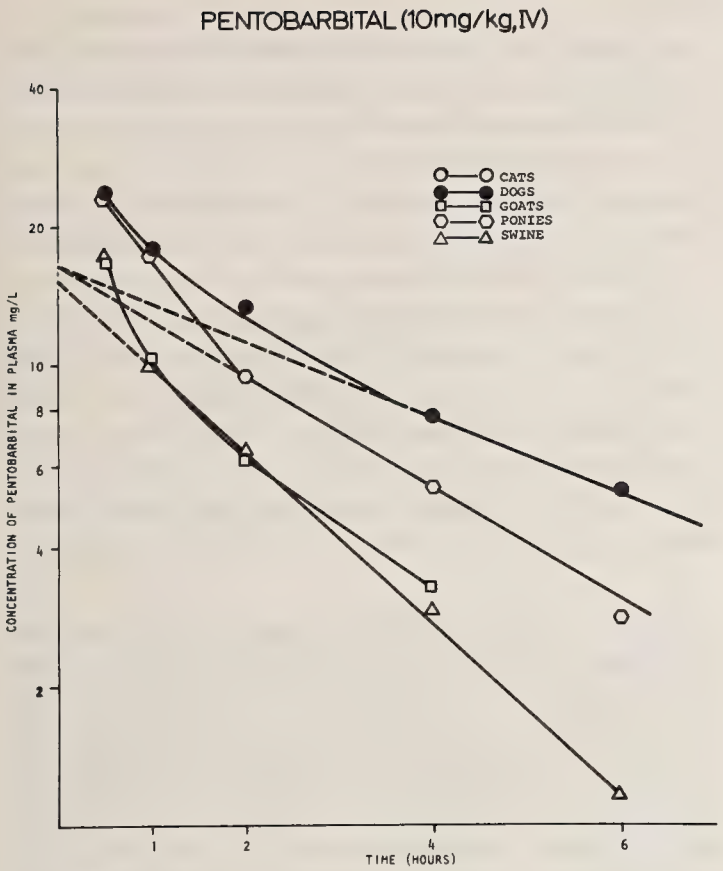


FIGURE 18.—Disappearance of pentobarbital from the plasma of several species following intravenous administration (10 mg/kg).

with oxidative mechanisms. For example, cats were quite efficient in the transformation of meperidine (N-dealkylation) and phenylbutazone (aromatic hydroxylation). The primary importance of biotransformation to the problem of species differences was well-illustrated by the study of tetraethylammonium. This drug is not metabolized in the body and species differences in rate of elimination were not observed.

Factors other than biotransformation may be important determinants of species differences in disposition of some drugs. Species differences in plasma protein-drug interaction were found to be important in the case of quinidine and phenylbutazone. Difference in specific apparent volume of distribution was an important determinant of duration of action of pentobarbital. In general, basic drugs had large volumes of distribution indicative of sequestration in the body and acidic drugs had much smaller volumes of distribution.

The results of this investigation illustrate the futility of attempting to extrapolate information derived from one species of animal to another species. We would conclude on the basis of our experience that any drug should be evaluated in the animal species in which it is to be used rather than attempt to apply information obtained in one species to another. Thus, the ultimate aim of this investigation was not attained. I don't believe that it is possible to develop an animal system which would predict the implications of drug therapy in man.

REFERENCES

1. BRODIE, B. B. Clinical implications of drug metabolism. Proc. Mid-Year Meeting, Amer. Pharmaceut. Manufact. Assn., 122–143, 1952.
2. WILLIAMS, R. T. *Detoxication Mechanisms.* 2nd ed. John Wiley & Sons, N. Y. 1959.
3. DONE, A. K. Developmental pharmacology. Clin. Pharmacol. Therap. 5:432, 1964.

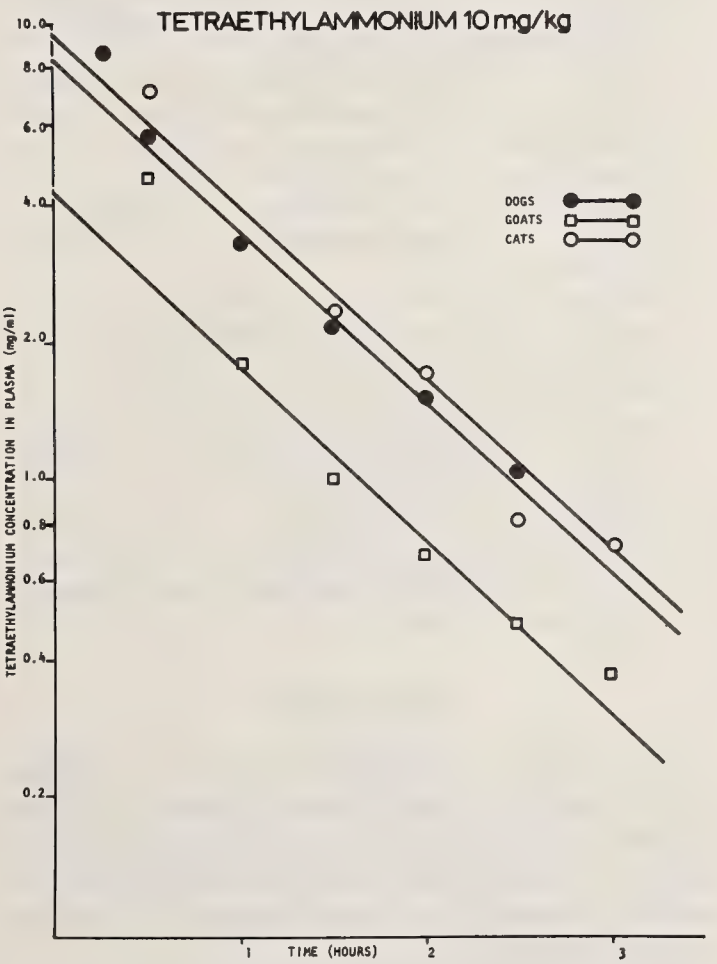


FIGURE 19.—Disappearance of tetraethylammonium from the plasma of dogs, cats and goats following intravenous administration (50 mg/kg).

4. Saltzman, A. Fluorophotometric method for the estimation of salicylate in blood. J. Biol. Chem. 174:399, 1948.
5. Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., and Papper, E. M. The fate of pentobarbital in man and dog and a method for its estimation in biological material. J. Pharmacol. Exper. Therap. 109:26, 1953.
6. Burns, J. J., Rose, R. K., Chenkin, T., Goldman, A., Schulert, A., and Brodie, B. B. The physiological disposition of phenylbutazone in man and a method for its estimation in biological material. J. Pharmacol. Exper. Therap. 109:346, 1953.
7. Oehme, F. W. *A Comparative Study of the Biotransformation and Excretion of Phenol.* Ph.D. dissertation. University of Missouri, Columbia, Mo., 1969.
8. Davis, L. E., and Sturm, B. L. Drug effects and plasma concentrations of pentazocine in domesticated animals. Am. J. Vet. Res. 31:1631, 1970.
9. Glazko, A. J. Colorimetric procedure for the determination of chloramphenicol and related nitro compounds. Instructions obtained from the Research Laboratories, Parke Davis Company, Detroit, Michigan.
10. Cramér, G., and Isaksson, B. Quantitative determination of quinidine in plasma. Scandinav. J. Clin. Lab. Investig. 15:553, 1963.
11. Noonan, J. S., Murdick, P. W., and Ray, R. S. A method for detecting amphetamine using gas chromatography of a halogenated derivative. J. Pharmacol. Exper. Therap. 168:205, 1969.
12. Doluisio, J. T., and Swintosky, J. V. Biopharmaceutics II. Pharmacokinetics. Am. J. Pharm. 137:175, 1965.
13. Ferrari, R. A. Differential distribution of pentazocine-^{3}H and cis-3-chloroallylnorpentazocine-^{3}H in the cat. Toxicol. Appl. Pharmacol. 12:404, 1968.
14. Berkowitz, B. A., Asling, J. H., Shnider, S. M., and Way, E. L. Relationship of pentazocine plasma levels to pharmacological activity in man. Clin. Pharmacol. Ther. 10:320, 1969.
15. Theodorides, V. J., DiCuollo, C. J., Guarini, J. R., and Pagano, J. F. Serum concentrations of chloramphenicol after intraruminal and intra-abomasal administration in sheep. Am. J. Vet. Res. 29:643, 1968.

DISCUSSION

Bill Sacco, Biomedical Laboratory, Edgewood Arsenal: Even though I agree with your paper's last statement, this is done every day. So, if one has to make the inferred application safe to humans, what method would you suggest at the moment?

Dr. Davis: Well, I think it's quite discouraging. I think we were quite sanguine when we began this quest and the more we've learned, the more confusing the picture has become. If I had to make a judgment, I would think that the pig would be the most rewarding animal to look at. For many of these drugs, when we compare our values to those that are reported in normal humans, the pig, I think, gives the right answer more times than the other species. Obviously, the herbivorous animals are totally different in their handling of drugs than man. But I think Dr. Baggot pointed this out very well. Man seems to be unique, at least among the mammals that have been studied; there's just no accounting for man as far as mechanisms for biotransformation and so on. I just can't make generalizations at this point. It's very confusing.

Tibor Balazs, Food & Drug Administration, Washington, D.C.: I would like to stretch your final sentence. I might go to the extreme and say that it is very difficult or impossible to foresee the tentative behavior of a compound just from one individual to the other. That's what Dr. Cass so wisely pointed out after the previous talk. I have had such an experience with a number of drugs. Let's say I could find some dogs with phenylbutazone plasma level which was as flat as the cat salicylate plasma level in your second slide, and another dog in which phenylbutazone disappearance was as steep as salicylate disappearance in the plasma for the goat, for exmple, one could only expect consistency within each animal. In a study with Dicumarol, I measured biological activity (the pharmacologic hyperproteinemia), and I found that the effects were very consistent with repetition of tests for each animal.

The other aspect, as was so wisely pointed out, is that maybe some of these factors which govern the pharmacokinetic activity are subject to change. Let's say I can visualize that the cat might "learn" to metabolize a given drug in a different way.

Dr. Davis: Well, I'm certainly not trying to indicate that we should just get out of animal studies as far as drugs are concerned; this is obviously impossible. I would simply, though, substantiate Dr. Brody's position. That is, in the course of development of new drugs, I think it is important to get them into the species in

which they are going to be used as soon as the investigator can safely do so. And what I am thinking of is the possibility of coming up with a model in which we can get data comparable to what can be gotten by the clinical pharmacologist in a hospital. This is what we were hoping to do when we started out five years ago, but we certainly didn't do this, I'm afraid.

CHAIRMAN: Dr. Davis, I'd like to ask you one question that relates to your study and I wonder if you have any information on it. You have very clearly shown that there are species' difference in handling of drugs in terms of plasma levels after administration. Have you found any histologic differences in your studies between species, when one looks at organ toxicity?

The implications of my question are clearly related to drug testing prior to the use in man. We're all familiar with drugs that never got to the testing phase in man because in the test species there were histological changes in—let's say, the liver. Do you have any evidence that there are species' differences based on a *higher* plasma concentration that perhaps should not be extrapolatable to man?

DR. DAVIS: No, I think predominantly of the salicylates. In using our system, some or many of these drugs were studied chronically. For example, in the case of salicylates, we had animals that were receiving daily doses over a period of six months, and in no case did we see any problem from the salicylate in the equine or the caprine species. In the case of swine, most of these animals were allowed to breed and go through to term pregnancy and so on. In the case of the swine we did notice that the newborn piglets were bleeders. If we hadn't had a good animal caretaker, I'm convinced many of these would have died at birth simply from hemorrhage. This has been reported in man, as a problem. In the case of cats, we did see toxicity and in veterinary medicine this is a fairly important clinical problem. Unfortunately, much of the lay population considers aspirin to be innocuous because they take it by the handsfull. As soon as their cats get sick, they start giving them aspirin and then the veterinarian has trouble.

THE ACTIONS OF CARDIOACTIVE DRUGS ON DEVELOPING MYOCARDIUM

William F. Friedman*

Clinicians have long recognized age related differences in cardiac pharmacology. These phenomena may reflect altered drug matabolism or disposition in the immature organism and/or an altered sensitivity of fetal and neonatal myocardium per se. The latter possibility was examined by studying the responsiveness to cardioactive drugs of heart muscle isolated from a total of 50 fetal, newborn, and adult sheep. Fetal and adult cardiac muscle was equally responsive to isoproterenol and acetylcholine. Fetal heart was supersensitive to the positive inotropic effects of norepinephrine. At all ages acidosis attenuated the augmentation of contractility produced by norepinephrine. Propranolol exerted a more profound negative inotropic action on fetal than adult heart although the effectiveness of beta adrenergic receptor blockade by propranolol was equal in fetal and adult myocardium. Cardiac NE concentrations were significantly lower in the fetal than the newborn lamb ($P<0.001$), and highest in the adult ($P<0.002$). The activities of intraneural enzymes involved in NE biosynthesis and degradation, tyrosine hydroxylase and monoamine oxidase, respectively, were lowest in fetal heart, higher in the newborn, and highest in the adult ($P<0.001$), whereas the activity of catecholo-o-methyl transferase, an extraneural degradative enzyme, was highest in the fetus. Histochemical studies showed sparse catecholamine-containing neurons in fetal myocardium and a progressive increase of these nerves with age. In contrast, the extent of parasympathetic innervation of the fetal and adult hearts appeared similar. Thus, biochemical, pharmacological, and histochemical techniques describe differential development of parasympathetic and sympathetic innervation in the mammalian heart, and allow an explanation for age-dependent differences in autonomic responsiveness to the adrenergic, but not the cholinergic, neurotransmitter. It is apparent that a marked age-dependency of the myocardial responses to many cardioactive drugs exists that must be considered in any clinical evaluation of cardiac pharmacology in the perinatal period.

* Associate Professor of Pediatrics and Medicine Chief of Pediatric Cardiology University of California, San Diego School of Medicine La Jolla, California.

Recipient of Research Career Development Award #5 KO4 HE41737 from the National Heart and Lung Institute.

** Study Supported by U.S. Public Health Service Grant HE 12373.

INTRODUCTION**

Although it is common pediatric knowledge that altered responsiveness to drugs may be a function of the age of the recipient, to date, cardiovascular pharmacology in the perinatal period has been based almost entirely on empirical observations and extrapolations from known effects in adult patients. If age related differences exist in cardiac pharmacology, they could, of course, reflect altered drug metabolism or disposition in the immature organism. Certainly, too, the developing myocardium may differ from the adult in its sensitivity to cardioactive stimuli and it is to this question that the present investigations were directed. In order to study the myocardial responsiveness to drugs, atrial and ventricular heart muscle was isolated from fetal and neonatal lambs and adult sheep and studied in a myograph under identical conditions. The dose-effect relationships to norepinephrine, isoproterenol, propranolol and acetylcholine were analyzed, and form the basis for this report. In addition, biochemical and histochemical methods were employed in efforts to understand the observed age-related differences in pharmacologic responsiveness. Let us first examine certain aspects of developmental autonomic pharmacology.

PHARMACOLOGICAL STUDIES

Thus far, physiologic and pharmacologic studies undertaken to assess the maturation of the autonomic control of the circulation have been concerned largely with the ability of young animals to respond to various physiologic stimuli, such as hypoxemia, acidosis, and carotid sinus hypotension, or to the injection of catacholamines.[1-5] Although the autonomic nervous system plays an important role in car-

diocirculatory control in the mature mammal, the precise mechanisms and degree of neural control of the circulation of the fetus and newborn is not clear. There remains a lack of quantification of the development of the separate factors constituting an integrated circulatory response—the afferent, central, and efferent components of a vascular reflex, the responsiveness of the peripheral vasculature, and the direct inotropic and chronotropic effects on the myocardium of the sympathetic and parasympathetic neurotransmitters. The studies described in the present report were designed to define more clearly the myocardial responsiveness to the sympathetic and parasympathetic neurotransmitters.

In the past, a variety of technical difficulties have surrounded efforts to analyze myocardial contractility in the fetal or neonatal heart in situ. Thus, there is an absence of meaningful comparisons of fetal, neonatal and adult cardiac tissue to the sympathetic neurotransmitter, norepinephrine, and to the parasympathetic neurotransmitter, acetylcholine. We believe we have obviated many of these technical difficulties in our laboratory by isolating myocardium from fetal and newborn lambs and adult sheep, and studying its pharmacological responsiveness to the adrenergic and cholinergic neurotransmitters in a myograph under identical conditions. Other pharmacologic stimuli have also been employed to further understand age-related differences in responsiveness. In our studies, a single right ventricular papillary muscle or moderator band was removed rapidly from the heart of either a fetal lamb in the last 15 days of gestation by hysterotomy under local anesthesia, or from a newborn lamb or an adult sheep, and suspended in a myograph in oxygenated Ringer's solution at 30°C, pH 7.4, and stimulated at 12 contractions/minute. The muscle was stretched to the apex of its length-active tension curve, and the changes in isometric tension were recorded after addition to the bathing medium of the appropriate pharmacologic stimulus. In appropriate experiments, atrial tissue rather than ventricular myocardium was studied in a similar manner.

Norepinephrine

Figure 1a shows the average dose response curves of fetal and adult ventricular myocardium to norepinephrine. It is quite clear that fetal cardiac tissue has a much lower threshold to the inotropic effects of norepinephrine, the sympathetic neurotransmitter, and is also about three-fold more sensitive to norepinephrine throughout the dose response curve when compared to the adult. Figure 1b shows a similar but not as marked contrast in sensitivity to norepinephrine when ventricular myocardium from newborn lambs younger than three days of age was compared to that obtained from lambs ranging in age from 3 days to 3 weeks.

Supersensitivity to norepinephrine would be expected if the fetal and early newborn myocardium lacked a complete development of sympathetic innervation, or if sympathetic nerves within these young hearts had a reduced capacity to take up and bind catecholamines. Uptake and binding represent an important mechanism for the inactivation of norepinephrine, and it would be anticipated that a reduction in the heart's ability to utilize this mechanism of inactivation would permit a higher concentration of the neurotransmitter to reach and activate the heart's beta receptors, and thereby render the tissues supersensitive. In order to more completely evaluate this hypothesis, the phamacologic responsiveness of fetal and adult ventricular muscle to isoproterenol was determined.

Isoproterenol

Isoproterenol is a direct acting beta adrenergic agonist that is not taken up and stored in sympathetic nerves. In contrast to the findings with norepinephrine, equal responsiveness of fetal and adult myocardium was observed (Figure 2). This finding indicates that beta receptor sensitivity to catacholamines is similar in adult and fetal myocardium, and supports the hypothesis that sympathetic innervation is incomplete or functionally immature in the heart of the fetus or early newborn.

Acetylcholine

Vagal stimulation or administration of the parasympathetic neurotransmitter, acetycho-

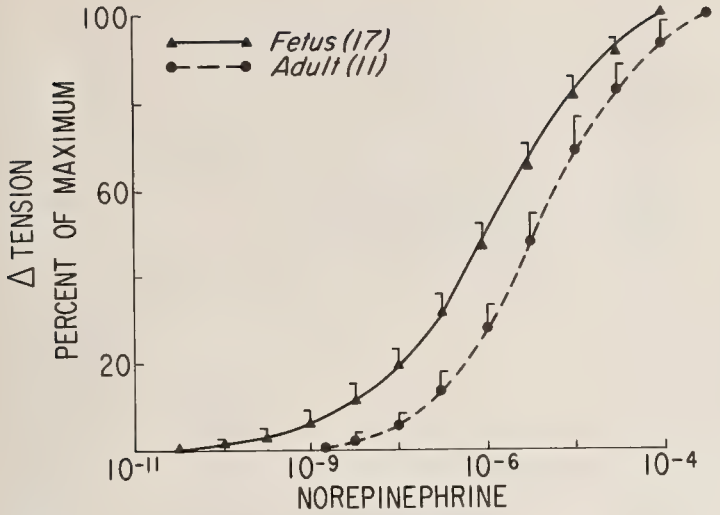
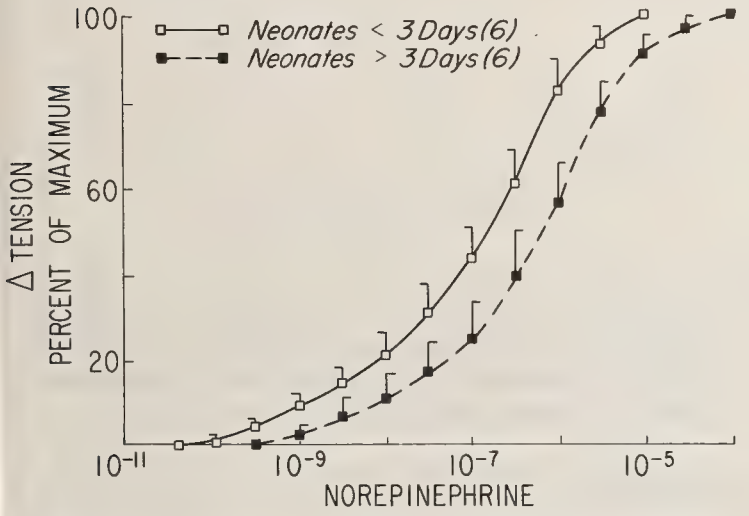


FIGURE 1.—a) Average norepinephrine dose response curves of isolated right ventricular myocardium obtained from 17 fetal lambs and 11 adult sheep. Vertical bars: S.E.M.



b) Average norepinephrine dose response curves of isolated right ventricular myocardium obtained from 6 newborn lambs less than 3 days of age and 6 newborns ranging in age from 3 days to 3 weeks. Vertical bars: S.E.M.

line, depresses atrial contractions. Figure 3 illustrates our data concerning the effects of acetylcholine on myocardial contractility. These dose response curves demonstrate that the negative inotropic effects of acetylcholine on isolated atrial myocardium is similar in the fetal lamb when compared to the adult sheep. Furthermore, when the atria are pre-treated with atropine the responsiveness to acetylcholine is different at the two different ages. In this circumstance, we are looking at the composite result of a number of acetylcholine effects. First the muscarinic depression of contractility which is inhibited competitively by atrophine. Secondly, in high concentrations, acetylcholine may produce a positive inotropic effect by releasing catacholamines from sympathetic nerve endings in the heart. The separation after atropine of the fetal acetylcholine dose response curve from the adult curve may be another reflection of reduced or functionally immature sympathetic innervation in the fetus, and reduced norepinephrine release by acetylcholine.

Acidosis

It has been suggested that acidosis does not depress cardiac function in the newborn animal in the presence of an intact sympathetic nervous system unless hypoxemia supervenes.[5,7] Figure 4 is representative of a number of experiments in which force-velocity curves were constructed from isotonic, afterloaded contractions at a high pO2 before the addition of norepinephrine at a normal pH, and at a reduced pH of 7.1. Acidosis was produced by either increasing the pCO2 in the muscle bath or by adding lactic or hydrochloric acid. Raising the pCO2, analogous to respiratory acidosis, always depressed contractility more than by simulating metabolic acidosis.

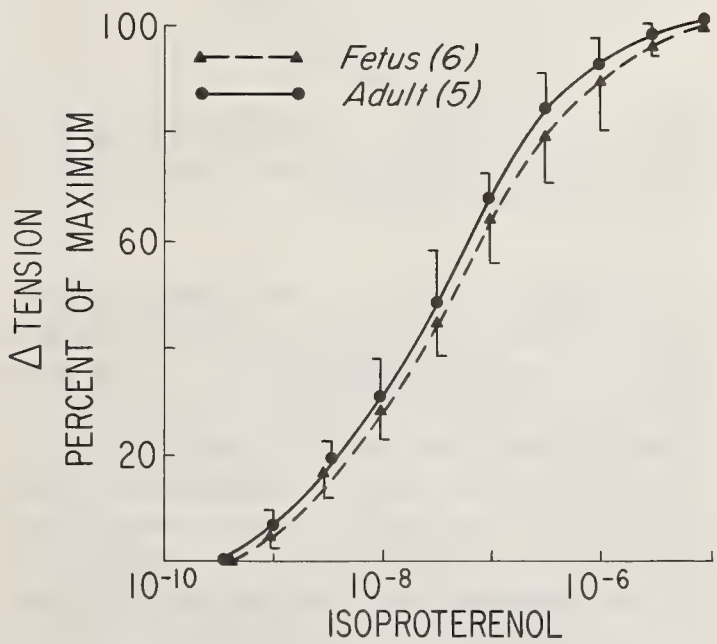


FIGURE 2.—Average isoproterenol dose response curves showing equal responses for fetal and adult ventricular tissue. The numbers in brackets are the numbers of animals from which cardiac tissue was obtained. Vertical bars: S.E.M.

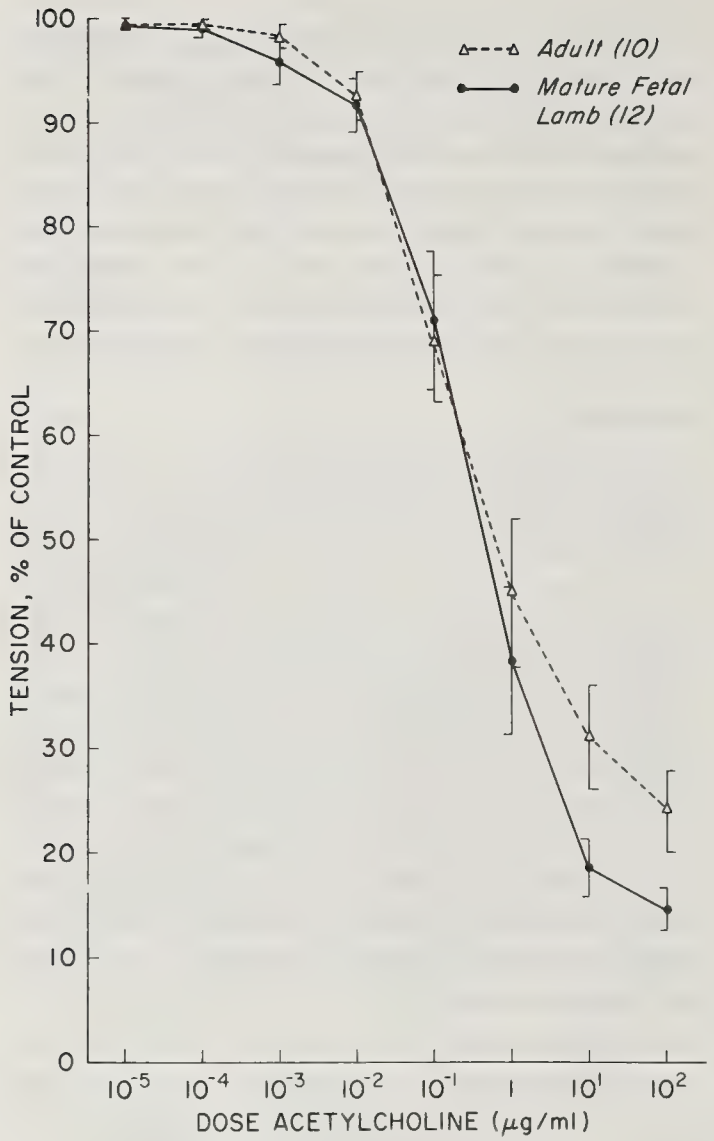
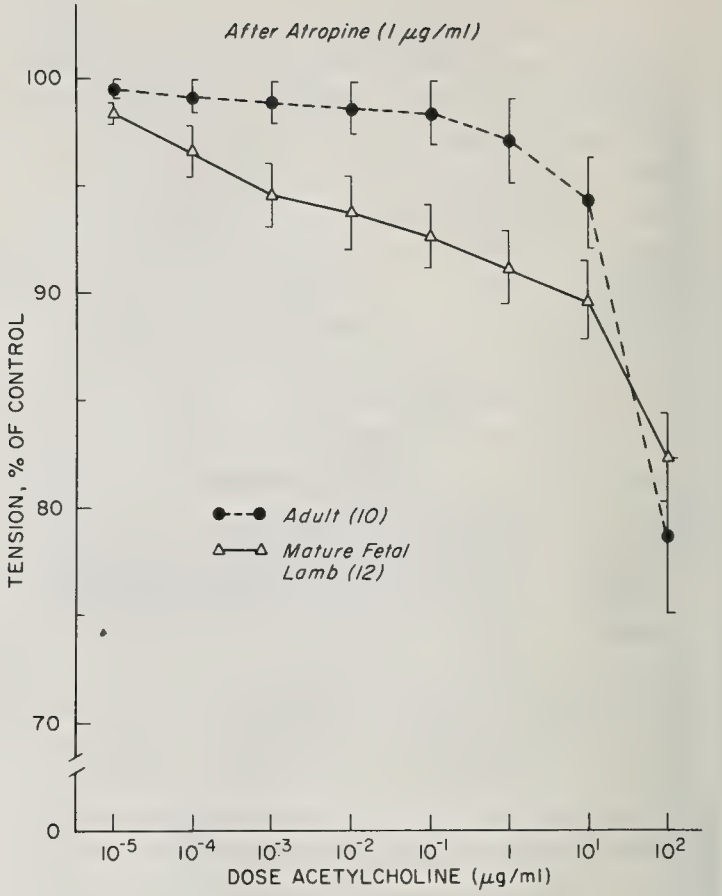


FIGURE 3.—a) Average acetylcholine dose response curves of isolated right atrial myocardium stimulated at 30 contractions per minute.



b) Average acetylcholine dose response curves of isolated right atrial myocardium after pre-treatment with atropine. The numbers in parentheses are the numbers of animals from which cardiac tissue was obtained. Vertical bars: S.E.M.

As Figure 4 illustrates, when the pH is lowered, the force-velocity curve is depressed and shifted downward and to the left. At all ages studied, for both normal and acidotic muscles, norepinephrine produced a marked increase in tension and in the velocity of shortening at any tension, and the absolute increase was similar under both conditions. However, despite this marked augmentation in contractility, when the pH was reduced, there was substantially less force generation and velocity of shortening than heart muscle at a normal pH. These findings indicate clearly that acidosis produces a shift in norepinephrine dose responsiveness, even in the absence of hypoxia.

Propranolol

Since age related differences in myocardial responsiveness to norepinephrine were observed it was considered important to examine the age dependency of the effects of a beta adrenergic *antagonist,* propranolol. Propranolol possesses other actions besides producing blockade of beta receptors.[6] Examination of the fetal curve in Figure 5 reveals, especially, the direct depressant effects of propranolol on the myocardium. This negative inotropic action is also seen in the adult curve, but in the adult propranolol exerts a small, direct, positive inotropic action before it depresses contractility. Thus, at any concentration of the beta blocker, the fetal heart is significantly more depressed than the adult. Despite this age-related difference in sensitivity to the inotropic effects of propranolol, we observed that the beta blocking action of the drug is the same for fetus and adult.

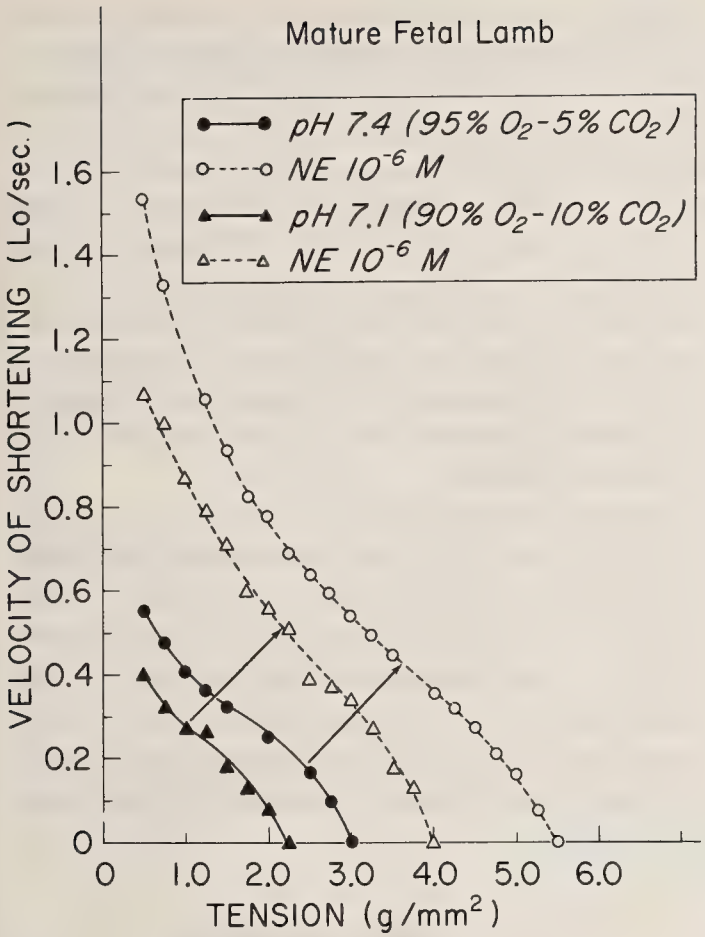


FIGURE 4.—A typical experiment in which force velocity curves were constructed from isotonic, afterloaded contractions of right ventricular myocardium isolated from a fetal lamb in the last week of gestation. The pH was reduced from 7.4 to 7.1 by increasing the carbon dioxide tension of the bathing medium. Acidosis depressed both velocity of shortening and tension development, and although norepinephrine augmented both velocity and force, the levels achieved were not comparable to those when the muscle was bathed at a normal pH.

Figure 6 shows dose-response curves of fetal and adult heart muscles to isoproterenol constructed in the absence of beta blocker (the curves labeled *control*) and in the presence of two different molar concentrations of propranolol (3×10^{-8} and 3×10^{-6}). As would be expected, propranolol reduced the response to isoproterenol in a dose-related manner and displaced the curves to the right. Thus, in the presence of beta blocker, a higher concentration of isoproterenol was required to achieve a given increase in tension. Note, however, that the degree of blockade was the same for the fetus and the adult.

Interestingly, in contrast to the age-dependent differences that existed in the inotropic actions of propranolol, we did not uncover a difference in the chronotropic actions of the beta blocker. A spontaneously beating SA node-right atrial preparation was employed to study the effects on intrinsic heart rate. In Figure 7 the absolute change in the frequency of contraction is plotted against the concentration of propranolol. Both the fetus and adult were slowed to the same extent.

BIOCHEMICAL STUDIES

These studies were undertaken to more completely understand the mechanism of the age-dependent differential sensitivity to norepinephrine described above. In order to describe the development of the heart's ability to synthesize, store and metabolize catacholamines, we have measured the myocardial norepinephrine stores and the critical enzymes in norepinephrine biosynthesis and degradatioon.

The cardiac concentration of norepinephrine in fatal, neonatal and adult animals may be employed as an index of the magnitude of sympathetic innervation because the heart's stores of

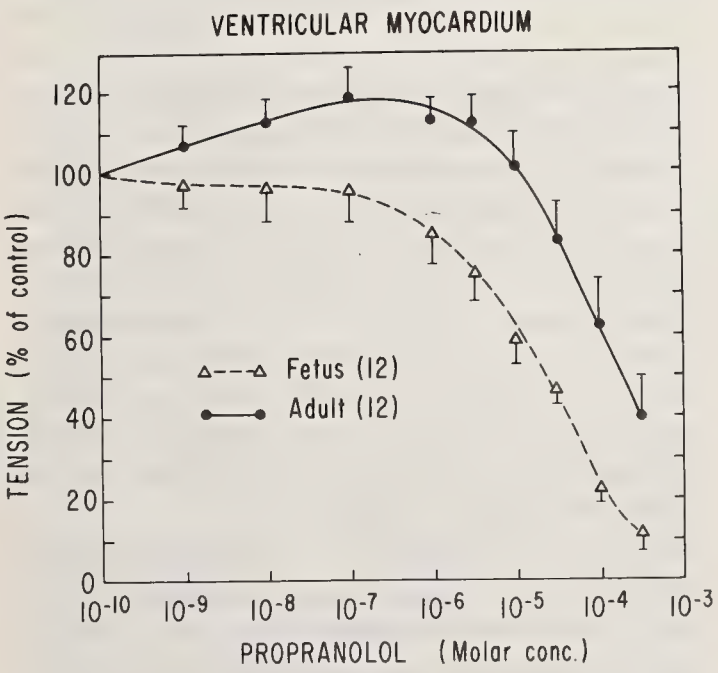


FIGURE 5.—Average propranolol dose response curves showing a more marked negative inotropic action on the fetal heart. The numbers in brackets are the numbers of muscles studied. Vertical bars: S.E.M.

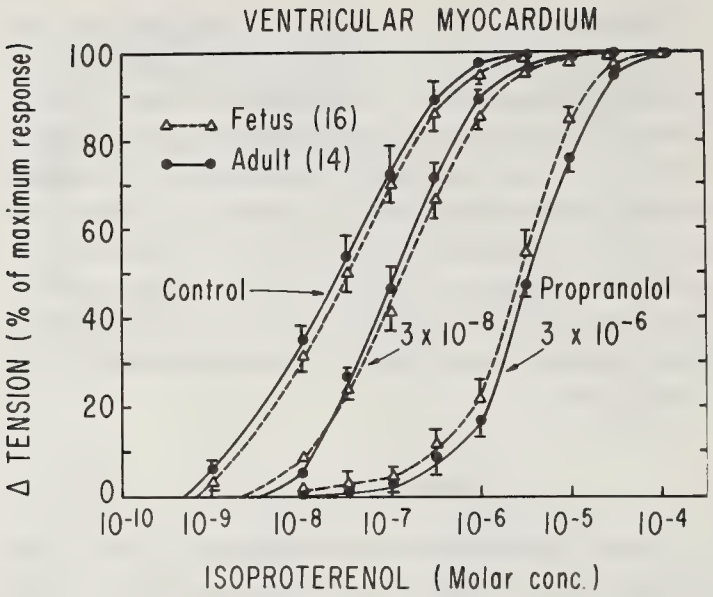


FIGURE 6.—Average dose response curve to isoproterenol in the absence and presence of different concentrations of propranolol. The beta blocking effect was comparable at both ages. The numbers in brackets are the numbers of muscles studied. Vertical bars: S.E.M.

norepinephrine are localized almost exclusively in intracellular storage sites within the terminations of the sympathetic nerves.[8] Norepinephrine was determined spectrophotofluorometrically by the trihydoxy indole method after alumina absorption.[9] Figure 8 compares the cardiac norepinephrine stores in lambs and sheep at different stages of development. Myocardial concentrations of norepinephrine in the fetus within several weeks of term was significantly lower than in the newborn lamb less than 3 days old, and there was no difference between older newborns and adult sheep. Similar results were observed when other mammalian species were studied. Thus, a gradual increase with advancing age was observed in the myocardial norepinephrine stores of the rabbit (Figure 9) and substantial age-related differences were also observed in swine (Figure 10).

The rate of formation of norepinephrine is determined by tyrosine hydroxylase. This intraneuronal enzyme catalyzes the first transformation in catacholamine biosynthesis.[10] Its activity was determined by measuring the formation of tritiated water from 3,5-ditritiotyrosine.[11] In the lamb and sheep tyrosine hydoxylase activity was lowest in the fetus, somewhat higher in the neonate, and highest in the adult (Figure 11). A significant correlation existed between tyrosine hydroxylase activity and the cardiac stores of norepinephrine at all of the age levels studied. It was of particular interest that the adrenal glands of these same animals showed equal activities of tyrosine hydroxylase and abundant catacholamine stores at all ages (Figure 12). These findings suggest that the low myocardial activity of the enzyme is related to delayed development of sympathetic innervation rather than to a generalized immaturity of enzyme synthesis.

Monoamine oxidase is a catabolic enzyme that catalyzes the oxidative deamination of norepinephrine, and the enzyme is located in part within sympathetic nerves. When this enzyme's activity was determined by measuring the deaminated 14C metabolites of 14C-tryptamine[12], we observed a reduced activity in the fetal heart when compared to the adult (Figure 13).

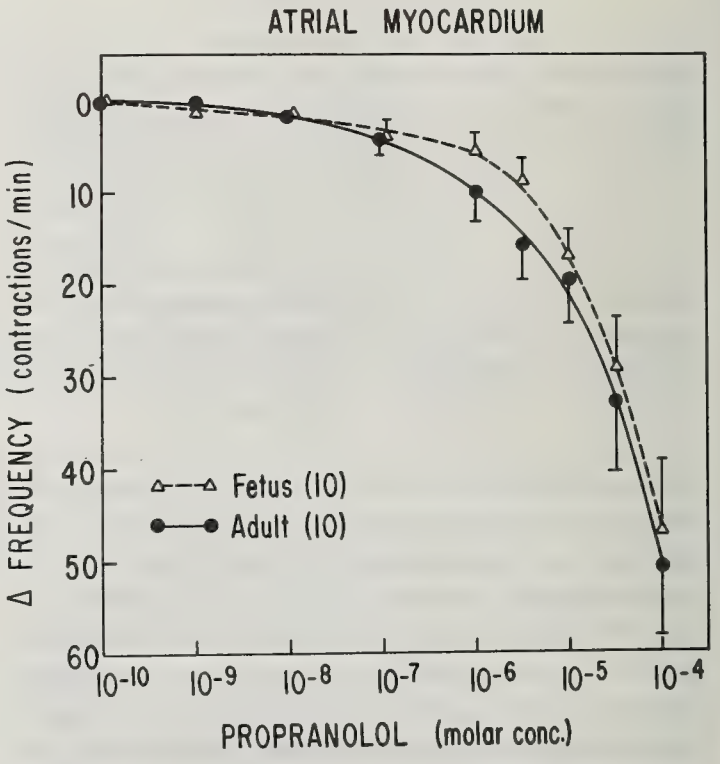


FIGURE 7.—Average curves obtained from studying spontaneously beating SA node-right atrial preparations showing no age-related differences in the negative chronotropic response to propranolol. Numbers in brackets are the numbers of atria studied. Vertical bars: S.E.M.

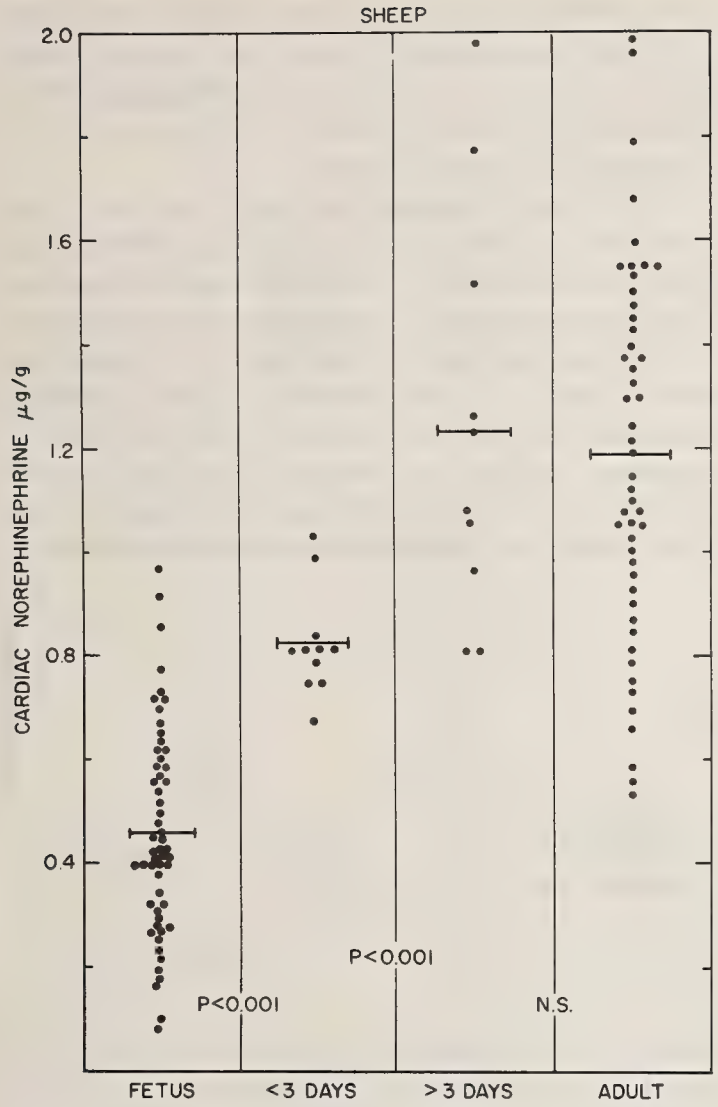


FIGURE 8.—Myocardial norepinephrine concentrations at different stages of development in the lamb and sheep. Horizontal bars: mean values.

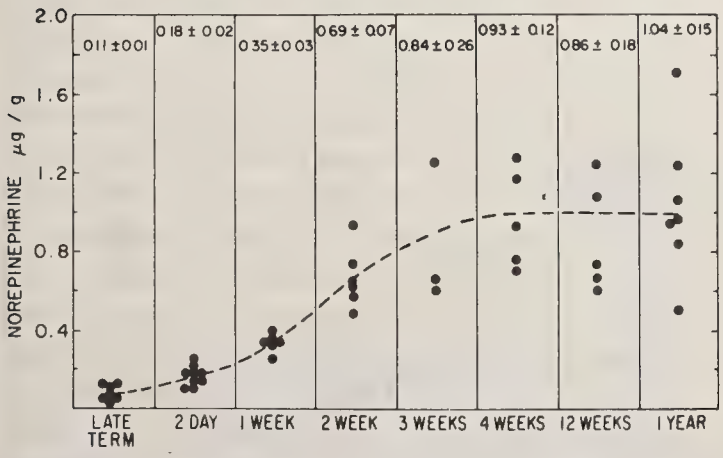


FIGURE 9.—Gradual increase with advancing age in the myocardial norepinephrine stores in the rabbit. Mean values are joined by the dashed line.

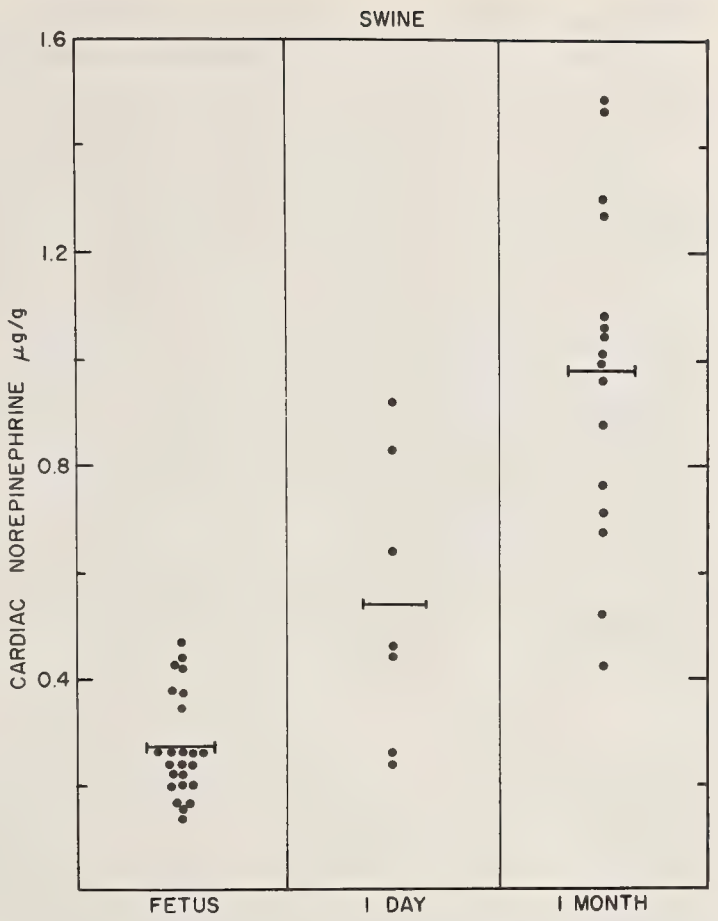


FIGURE 10.—Myocardial norepinephrine concentrations at different stages of development of the swine. The fetal values were obtained within 2 weeks of term. Horizontal bars: mean values.

In contrast to norepinephrine and the intraneuronal enzymes, tyrosine hydoxylase and monoamine oxidase, catachol-o-methyl transferase metabolizes norepinephrine outside the nerve only. The activity of catachol-o-methyl transferase was determined by measuring the 14C-normetanephrine formed from norepinephrine and 14C-S-adenosylmethionine in the presence of divalent cations.[12] The activity of this enzyme was significantly higher in fetal than in adult myocardium (Figure 14) and significant inverse relationships were evident when the catachol-o-methyl transferase activity was correlated with cardiac norepinephrine concentration, tyrosine hydroxylase activity, and monoamine oxidase activity.

All of the above biochemical observations are compatible with the concept that a significant increase in the magnitude of sympathetic innervation occurs post-natally.

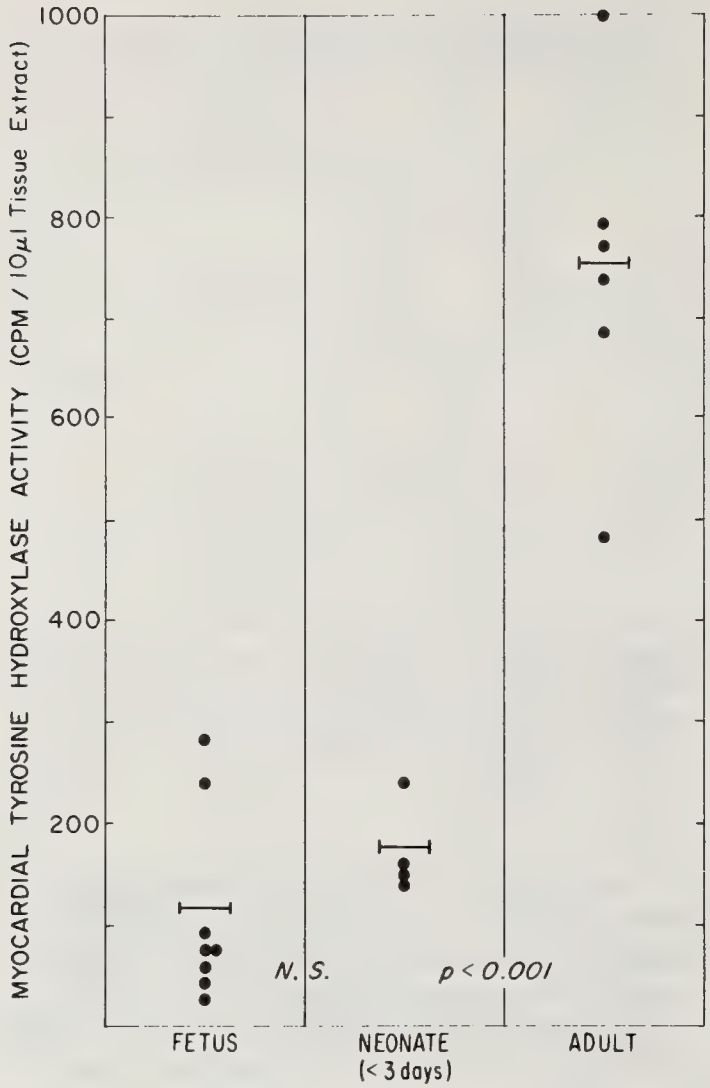


FIGURE 11.—Myocardial tyrosine hydroxylase activities at different ages in the lamb and sheep. Horizontal bars: mean values.

HISTOCHEMICAL OBSERVATIONS

The monoamine fluorescence technique of Falck and Owman[13] was employed to evaluate histochemically the distribution of sympathetic nerves within the hearts of the different aged animals. These studies provided final confirmation that sympathetic innervation is incomplete in the fetal and early newborn heart, or that uptake and binding are immature. In these studies the myocardium is treated in such a way that the norepinephrine within the adrenergic nerves fluoresces and the fluorescence is visualized microscopically. As Figure 15 shows, in the adult the heart is innervated by a dense network of intensely flourescent, varicose nerve fibers. At all ages, there appear to be more adrenergic fibers in the atria than in the ventricles, but more importantly, there is a reduced density of neurons in both fetal chambers when compared to the adult. Similar findings were observed in the rabbit heart.[14]

In both the lamb and the rabbit, the fetal myocardium contained large, intensely flourescent, preterminal nerve trunks in the epicardium and the tunica adventitia surrounding coronary arteries, and coursing in the connective tissue between cardiac muscle bundles. In these younger animals, intense flourescence resided only in terminal varicosities within the large nerve trunks. In contrast, the nerve trunks in the older sheep or rabbits did not contain terminal

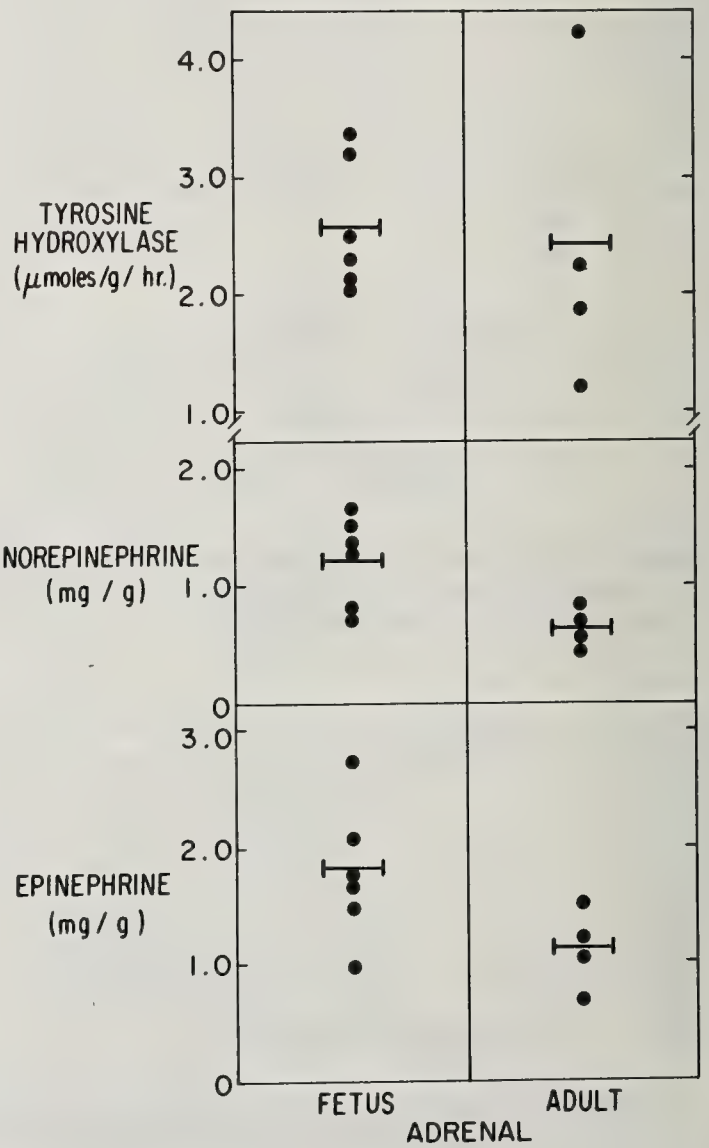


FIGURE 12.—Adrenal tyrosine hydroxylase activities and norepinephrine and epinephrine concentrations in the fetal lamb and adult sheep. Horizontal bars: mean values.

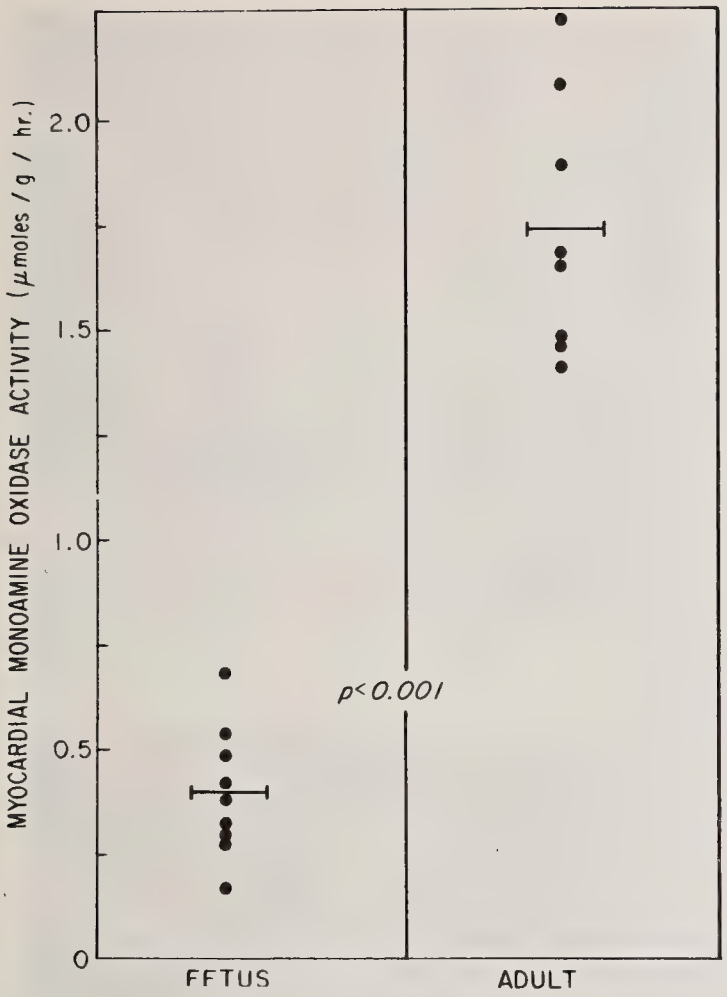


FIGURE 13.—Myocardial monoamine oxidase activities in the fetal lamb and adult sheep. Horizontal bars: mean values.

varicosities and flouresced only weakly. The appearance of large preterminal nerve trunks enclosing intensely flourescent, catacholamine-containing terminal varicosities suggests that the latter move into the heart during early development, perhaps in response to an increase in sensory input, and branch out and arborize to form the autonomic ground plexus. Since norepinephrine storage granules flow down cardiac adrenergic nerves from neuronal cell bodies in the cervical and thoracic sympathetic ganglia, the intense preterminal flourescence suggests that these ganglia are quite prepared to respond appropriately to central efferent impulse traffic. Moreover, it appears that a significant proportion of the norepinephrine measured in the fetal and newborn heart resides in the preterminal nerve trunks, and therefore may not be in close anatomic proximity to the adrenergic receptors of the myocardial cell.

While one cannot stain specifically for acetylcholine in tissues, it is possible to localize acetylcholinesterase histochemically, and it has been shown that reasonably good correlations exist between the concentrations of acetylcholine, acetylcholinesterase, and choline acetylase in various regions of the peripheral and central nervous systems. The photomicrograph shown in Figure 16 has been stained for acetylcholinesterase by the thiocholine technique after selective inhibition of pseudocholinesterase.[15] It would appear that a similar density of stained cholinergic fibers is present in atrial, SA and AV nodal and ventricular tissue in both the fetus and the adult.

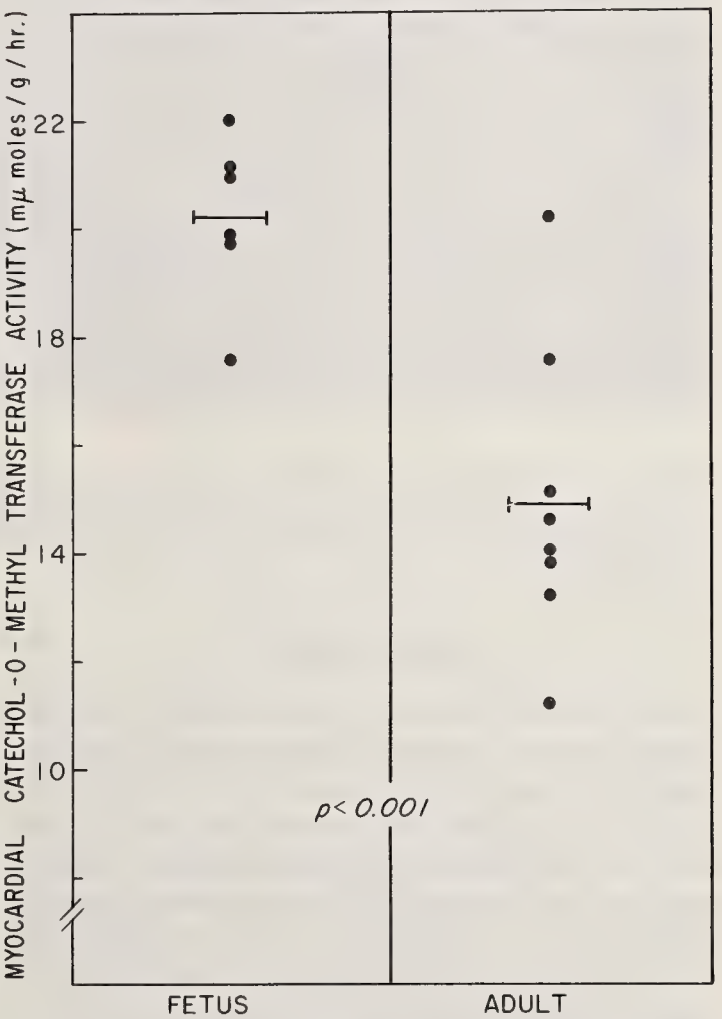


FIGURE 14.—Myocardial catachol-o-methyl transferase activities in the fetal lamb and adult sheep. Horizontal bars: mean values.

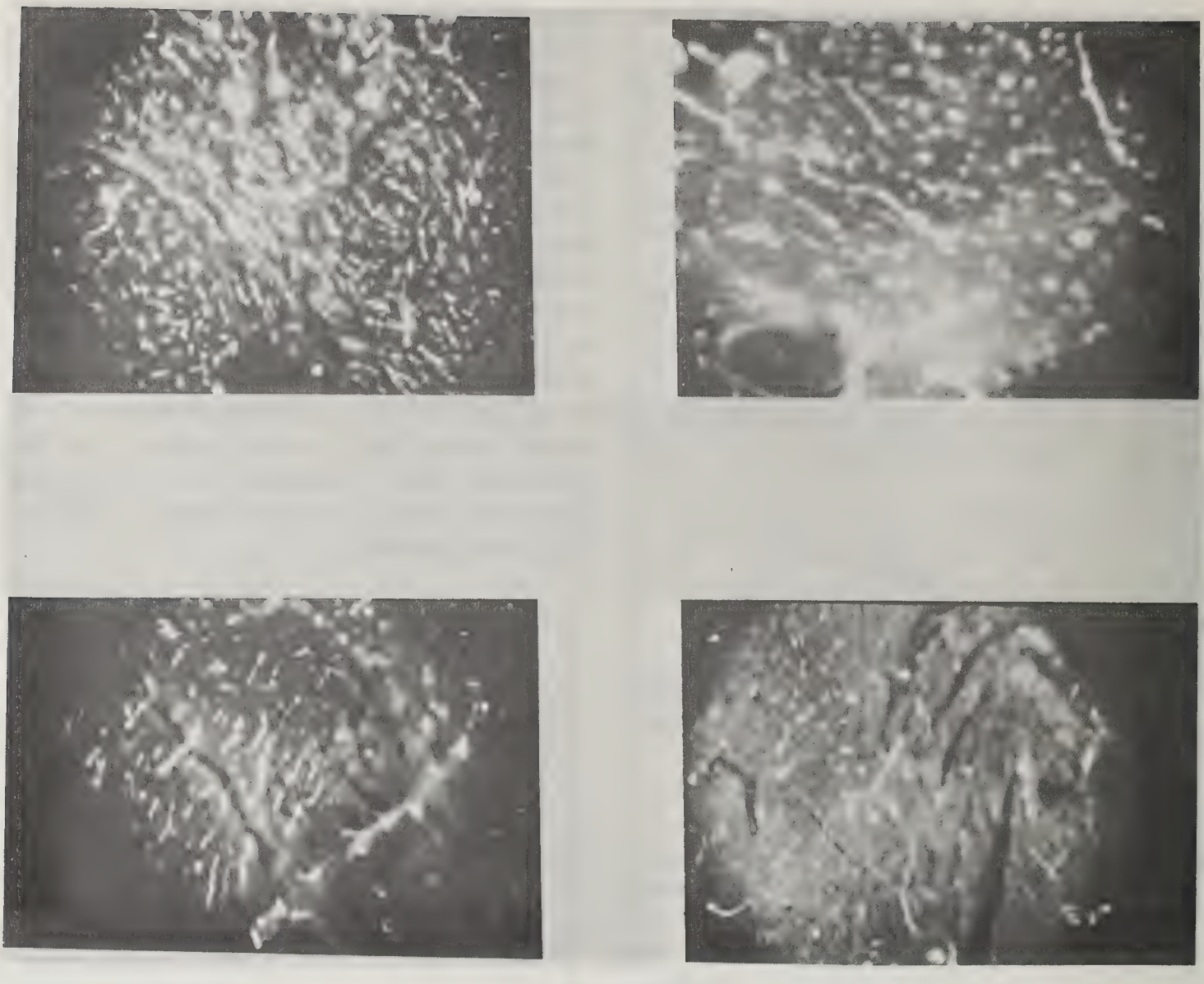


FIGURE 15.—Histochemical appearance (at the same magnification) of atrial (left) and ventricular (right) myocardium obtained from a 2-year old sheep (top) and a term fetal lamb (bottom). The Falck technique was employed to produce monoamine fluorescence of the heart's adrenergic nerve fibers. See text for further description.

CONCLUSIONS

The findings of these investigations illustrate that dynamic changes in the anatomic, biochemical and physiological disposition of cardiac catacholamines occur in the perinatal period and allow an explanation for age-dependent differences in the pharmacological responsiveness of ventricular myocardium to norepinephrine, the sympathetic neurotransmitter. The hearts of the fetuses close to term and youngest neonates were found to be supersensitive to norepinephrine. The histochemical findings supported the view that these hearts were partially innervated with a good deal of the visualized norepinephrine residing in preterminal nerve trunks rather than in terminal nerve endings. Beta receptor sensitivity was similar in fetal and adult myocardium, indicating that catacholamine receptor sites are fully functional before the complete development of an extrinsic nerve supply. The beta adrenergic antagonist, propranolol exerted a more marked direct depressant action on fetal heart when compared to the adult, but the degree of beta blockade was similar at both ages. At a comparable stage of development, the adrenal glands, unlike the heart, were found to

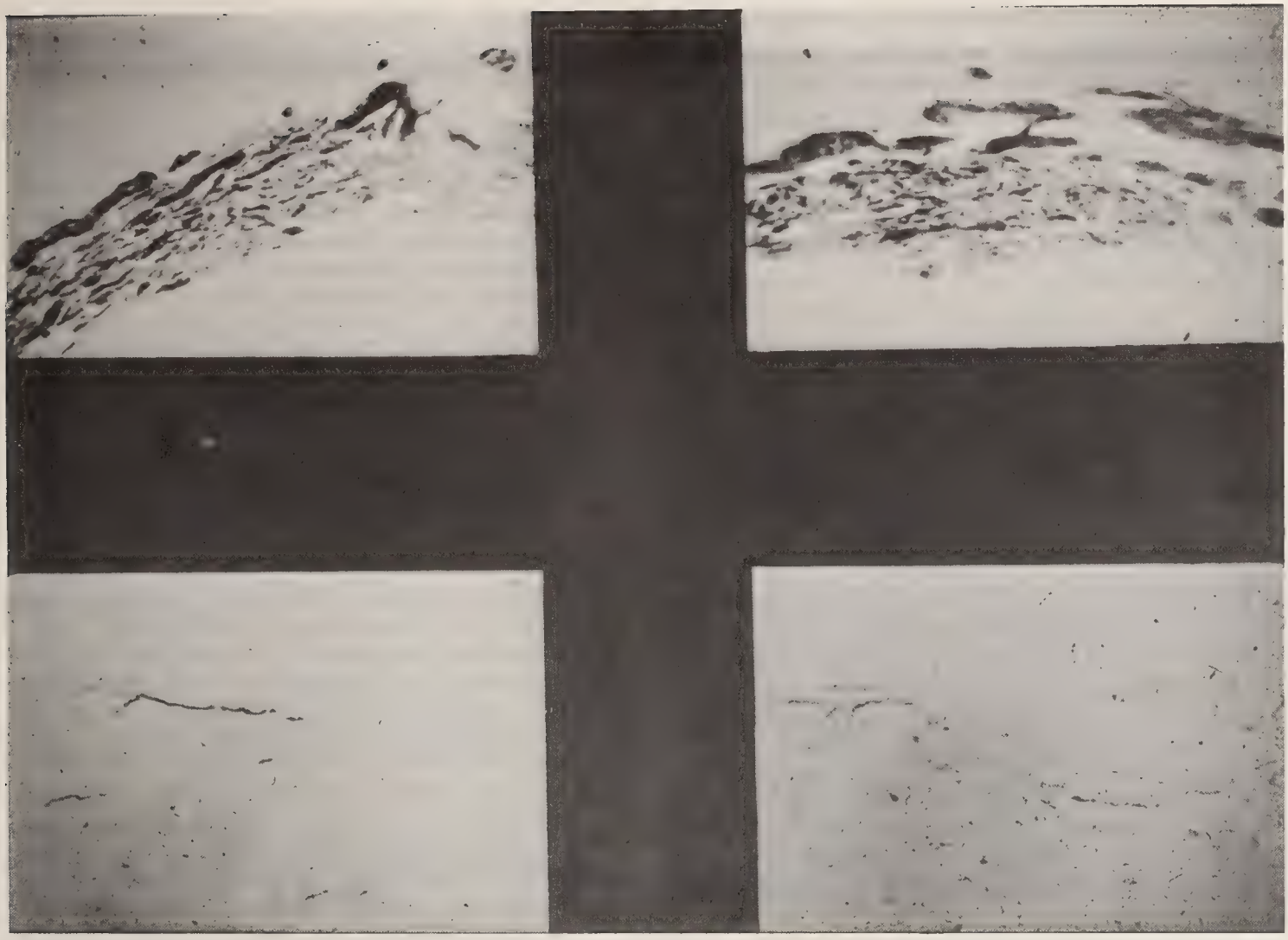


FIGURE 16.—Choloinergic fibers at the same magnification in the AV node (top) and interventricular septum (bottom) of the fetal lambs close to term (left) and adult sheep (right). No apparent age-related differences were observed.

contain abundant catacholamine stores. Moreover, at a stage in development when a reduction exists in sympathetic nerves and in cardiac norepinephrine stores and the intraneuronal enzymes concerned with norepinephrine biosynthesis and degradiation, there appear to be no age-related differences in parasympathetic innervation.

The heart of the adult mammal is richly supplied with sympathetic nerves and the release of norepinephrine from the endings of these nerves provides one of the fundamental mechanisms for the modulation of cardiac contractility.[16] Although there is evidence to suggest that regulation of the heart rate and arterial blood pressure in the newborn is under some autonomic control,[1-4] the extent of which sympathetic nerves contribute to the contractile state of the heart at this stage of development has not been previously defined. It has been shown that the ordinary activity of the adrenergic nervous system may have minimal effects on the normal heart, and that the intrinsic contractile state of the myocardium may not be influenced by alterations in endogenous catacholamine stores.[17] The force of contraction of the heart may be stimulated profoundly by an increase in the number of impulses traversing the sympathetic nerves whenever an imbalance exists between the cardiac output and the perfusion requirements of the peripheral tissues. When the latter occurs in the perinatal period, the interaction between a supersensitive myocardium and the adrenal release of catacho-

lamines may play a more critical, compensatory role in maintaining ventricular contractility than in the adult. In adult animals, it is evident that only a small fraction of the normal cardiac stores of norepinephrine is necessary to elicit a functional response to adrenergic nerve stimulation[18] or tyramine,[19] although the magnitude and perhaps the duration of the response may be reduced.[20]

SUMMARY

It is clear that a more complete understanding of the normal development of the heart and autonomic influences is necessary to provide a framework within which the effects of a variety of disease states can be elucidated. Further, it is apparent that in addition to looking for differences in drug metabolism and disposition in the immature organism the altered responses of fetal and neonatal myocardium per se must be considered in any evaluation of cardiac pharmacology in the perinatal period. Surely, it will be necessary to integrate analyses of all of these factors if we are to create a scientific framework for the rational, clinical approach to disease states encountered in the premature and newborn infant.

ACKNOWLEDGMENTS

A number of individuals have participated in a major way in various aspects of these studies, including Dr. Charles Cooper, Dr. David Jacobowitz, Dr. Peter Pool and Mrs. Shirley Seagren. I'm especially grateful to Dr. Eugene Braunwald for creating an environment in which the highest priorities exist for the advancement of the cardiovascular sciences.

REFERENCES

1. Downing, S. E., Milgram, E. A., and Halloran, K. H. Cardiac responses to autonomic nerve stimulation during acidosis and hypoxia in the lamb. Am. J. Physiol. 220:1956, 1971.
2. Downing, S. E., Gardner, T. H., and Solis, R. T. Autonomic influences on cardiac function in the newborn lamb. Circ. Res. 19:947, 1966.
3. Dawes, G. S., Handler, J. J., and Mott, J. C. Some cardiovascular responses in fetal, newborn and adult rabbits. J. Physiol. 139:123, 1967.
4. Born, G. V. R., Dawes, G. S., and Mott, J. C. Oxygen lack in autonomic nervous control of the fetal circulation in the lamb. J. Physiol. 134:149, 1956.
5. Talner, N. S., Gardner, T. H., and Downing, S. E. Influence of acidemia on left ventricular function in the newborn lamb. Pediatrics 38:457, 1966.
6. Parmley, W. W., and Braunwald, E. Comparative myocardial depressant and anti-arrhythmic properties of d-propranolol, dl-propranolol and quinidine. J. Pharmacol. Exptl. Therap. 158:11, 1967.
7. Downing, S. E., Talner, N. S., and Gardner, T. H. Influences of arterial oxygen tension and pH on cardiac function in the newborn lamb. Am. J. Physiol. 211:1203, 1966.
8. Dahlstrom, A., Fuxe, K., Mya-tu, M., and Zetterstrom, B. E. M. Observations on adrenergic innervation of dog heart. Am. J. Physiol. 209:689, 1965.
9. Crout, J. R., Creveling, C. R., and Udenfriend, S. Norepinephrine metabolism in rat brain, heart. J. Pharmacol. Exptl. Therap. 132:269, 1961.
10. Levitt, M., Spector, S., Sjoerdsma, A., and Udenfriend, S. Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea pig heart. J. Pharmacol. Exptl. Therap. 148:1, 1965.
11. Levitt, M., Gibb, J. W., Daly, J. W., Lipton, M., and Udenfriend, S. A new class of tyrosine hydroxylase inhibitors and a simple assay of inhibition in vivo. Biochem. Pharmacol. 16:1, 1967.
12. Krakoff, L. R., Buccino, R. A., Spann, J. F., Jr., and DeChamplain, J. Cardiac catachol-o-methyl transferase and monoamine oxidase activity in congestive heart failure. Am. J. Physiol. 215:549, 1968.
13. Falck, B. and Owman, C. A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta Univ. Lund. II: 7, 1965.
14. Friedman, W. F., Pool, P. E., Jacobowitz, D., Seagran, S. C., and Braunwald, E. Sympathetic innervation of the developing rabbit heart: biochemical and histochemical comparisons of fetal, neonatal, and adult myocardium. Circulation Res. 23:25, 1968.
15. Jacobowitz, D., and Koelle, G. B. Histochemical correlations of acetylcholinesterase and catacholamines in post-ganglionic autonomic nerves of the cat, rabbit and guinea pig. J. Pharmacol. Exptl. Therap. 148:225, 1965.
16. Braunwald, E., Chidsey, C. A., Harrison, D. C., Gaffney, T. E., and Kahler, R. L. Studies on the function of the adrenergic nerve endings in the heart. Circulation 28:958, 1963.
17. Spann, J. F., Jr, Sonnenblick, E. H., Cooper, T., Chidsey, C. A., Willman, V. L. and Braunwald, E. Cardiac norepinephrine stores and the contractile state of heart muscle. Circulation Res. 19:317, 1966.

18. ANDEN, N. E., MAGNEUSEN, T., and WALDECK, B. Correlation between noradrenaline uptake and adrenergic nerve function after reserpine treatment. Life Sci. 3:19, 1964.
19. CROUT, J. R., MUSKUS, A. J., and TRENDELENBURG, U. Effect of tyramine on isolated guinea pig atria in relation to their noradrenaline stores. Brit. J. Pharmacol. 18:600, 1962.
20. COVELL, J. W., CHIDSEY, C. A., and BRAUNWALD, E. Reduction of the cardiac response to post-ganglionic sympathetic nerve stimulation in experimental heart failure. Circulation Res. 19:51, 1966.

DISCUSSION

FRANCIS CAVOTO, Medical College of Pennsylvania, Phila.: You alluded to the fact that the rat also shows the same delay after birth in developing a sympathetic nervous system. What time schedule is that?

DR. FRIEDMAN: It was very similar to the rabbit curve. By about three to four weeks of age, the uptake of cardiac tritiated catecholamines, for example, are equivalent to the adult in the rat.

CHAIRMAN COHEN: Bill, do you have any idea what the stimulus is to change the fetal response to the adult after birth.

DR. FRIEDMAN: Thus far the answer to that would just be conjecture. We are now studying the chronically instrumented fetus where we can adjust conditions in utero and then see if, at term, we have altered such things as cardiac catecholamines. Currently, we are studying alterations in baroreceptor stimuli during fetal life with chronically implanted sonomicrometers on the endocardial surface of the left ventricle. The results are not yet in with regard to whether there will be a difference in the extent and distribution of sympathetic nerves at the time of birth because of all this sensory input during fetal life.

PITAMBAR SOMANI, Abbott Laboratories, North Chicago: You alluded to the fact that norepinephrine concentration in fetal heart is very low, and also that the enzymes for synthesis are quite low. Can you tell us whether innervation is complete at this point and is it only a question of lack of enzymes, or is the nerve development also incomplete at this point?

DR. FRIEDMAN: I can't be terribly specific. It would appear that all of this is a reflection of incomplete innervation, rather than a question of enzyme induction. That would be my guess. It is not corroborated by hard scientific data at this point.

DRUG STUDIES ON ISOLATED ANIMAL AND HUMAN CARDIAC AND VASCULAR TISSUES: ADVANTAGES AND LIMITATIONS

Joseph V. Levy*

The use of isolated cardiac and vascular tissues for characterizing the actions of drugs has long been held as a necessary and rational prelude to studies on the intact organism. However, there is an increasing awareness that important qualitative and quantitative differences exist between various animal species with regard to the efficacy and toxicity of various agents. Traditionally, a variety of animal cardiac and vascular tissues have been used in an attempt to predict the cardiovascular actions of agents in man. However, with the advent of open-heart surgery, another dimension has been added to the pharmacologist's methodology: The availability and use of fresh biopsied cardiac and vascular human tissue for in vitro drug studies. Our own research over the past 12 years has shown the feasibility of routinely obtaining viable cardiac and vascular tissues for drug and physiological studies in the laboratory. For example, our recent research has dealt with a characterization of the actions of beta-adrenergic blocking drugs, prostaglandin E_2, glucagon, ouabain, papaverine, aminophylline and other agents on isolated human atrial tissue. Comparisons have been made with the effects on rabbit atrial tissue preparations. Important qualitative and quantitative differences in human and animal tissue responses were noted with several of these drugs and substances. Isolated human saphenous veins also have been used as a model of human vascular tissue. Comparisons with animal vascular tissue have been made. Again, important qualitative and quantitative differences have been noted between human and animal vascular tissue responses to drugs. While the use of human tissues has added an important new tool to the pharmacologist's armamentarium of tests, caution must be exercised in interpreting results with such tissues because of known or unknown disease processes or uncontrollable conditions which can modify and influence in vitro drug responses. In one way this is a major limitation. On the other hand, the use of diseased tissues for drug studies might have more relevance in terms of predicting drug effects on intact man afflicted with this disease. A discussion of methods currently being used for in vitro studies also is presented.

* Laboratory of Pharmacology and Experimental Therapeutics, Pacific Medical Center, San Francisco, California.

INTRODUCTION

The purpose of this paper is to present a survey and discussion of the use of certain isolated animal and human tissues for characterizing the action of bioactive substances on the heart and blood vessels.

Of course, the use of isolated organs and tissues from various animals long has been held as a vital test system for characterizing the cardiac and vascular effects of a wide spectrum of natural and synthetic substances. In vitro models have become standarized and widely accepted as an important part of the pharmacologist's armamentarium in his search for new drugs, or characterizing the mechanism of action of agents already in therapeutic use. Yet, the major disadvantages and limitations of these convenient in vitro animal models too often have been ignored. Moreover, their usefulness and limits as models for predicting in vivo action in animals and man have not been fully appreciated.

In our own laboratory, we felt that while the classical in vitro animal tissue models were more often right than wrong in terms of assessing actions in vivo, the major exceptions caused us to develop in vitro systems for the routine and systematic study of isolated human tissues. Hopefully, the use of these isolated human tissues might add another dimension to our ability to predict cardiovascular drug efficacy or toxicity in man.

GENERAL PRINCIPLES

Before detailing specific examples of the advantages or limitations of animal and human in vitro tissue models, I think it is important to outline some basic principles which are often

TABLE I.—*Desired Conditions for Use of Animals Yielding Tissues for in Vitro Study*

1. Definition and knowledge of genotype.
2. Precise knowledge of morphological and physiological status of animal.
3. Absence of specific pathogenic agents, parasites, and disease states.
4. Standardized environmental conditions.
5. Knowledge of seasonal or circadian variations in function and reactivity.

forgotten by the physiologist and pharmacologist. First, there are questions relating to the animal source from which various isolated cardiac or vascular preparations are obtained. Table I lists some factors which one must consider in selecting any given species or strain of animal, and some known major factors that can determine the subsequent responses of isolated tissues obtained from these animals. While some of these factors have been known or appreciated for many years, others (e.g., the influence of circadian rhythm on drug responses) are only now being fully documented and investigated.

Depending on one's perspective, the goals of in vitro studies may vary. From the point of view of many pharmacologists, however, the main objective is to utilize those in vitro techniques which take advantage of the known diversity of function and morphology of any given tissue, derived from a variety of sources, and which increases our understanding of general principles of drug action. This then leads to the important questions of (1) what are the most suitable and meaningful in vitro preparations for the assessment of drug action, and (2) how valid are these in vitro tests in predicting effects in the intact organism?

Relative to the last question, many important positive correlations exist between in vitro tests and effects observed in man (e.g., the cardiotonic effect of cardiac glycosides). Yet major disturbing discrepancies exist which indicate we must be extremely cautious of extrapolating in vitro results to the intact animal. Table II shows three types of agents which produce qualitatively or quantitatively different results depending on the test system. Many more examples are available.

TABLE II.—*Examples of Poor Correlation Between in Vitro and in Vivo Drug Effects*

Agent	Effect	
	In vivo	In vitro
Eledoisin	Vasodilator	Vasoconstrictor
Prostaglandin E_2	Vasodilator	Vasoconstrictor
Ko-592	Weak β-blocker in the rat	Potent β-blocker

Some of these discrepancies would, of course, lead to the discarding or dismissal of many important agents, depending on which test might be utilized first. For example, in Table III, the inotropic effects of cyclic AMP and angiotensin would be underestimated by the use of the indicated in vitro tests because of permeability problems, or species/tissue differences. Similarly, the in vivo vascular effect of bradykinin would be missed if one utilized the tissues indicated. Clearly, the wider the spectrum of in vitro/in vivo testing, the less likely one would miss meaningful biological activity. Yet, are there any clues which allow us to rationally choose one test over another?

Table IV summarizes some selected studies indicating the diversity of results pertaining to the inotropic responses of isolated cardiac prep-

TABLE III.—*"False Negative" in Vitro Responses to Active Agents*

Agent	Tissue	Effect	Reason
Cyclic AMP	Heart	Weak inotropic	Permeability barrier
Bradykinin	Human Vein Strip Rabbit Aortic Strip	No contraction	Species/tissue
Angiotensin	Kitten atrium; Frog Vent.	Weak inotropic No contraction	Species/tissue

TABLE IV.—*Species Differences in Inotropic Responses to Prostaglandins*

Tissue*	PG	Species	Effect	Investigator**
L. Atrium	E_2	Rabbit	+	Levy
L. Atrium	E_2	Human	±; 0	Levy
Vent.	E_1	Cat	0	Berti et al.
Vent.	E_1	Rat	0	Berti et al.
L. Atria	E_1,A_1,F_2	G.P.	+	Nutter & Ratts
L. Atria	E_1,A_1,F_2	Rabbit	0	Nutter & Ratts
Vent.	E_1	Toad	+	Nutter & Ratts

* Isolated hearts
** See References
+ = positive inotropism
0 = no significant effect
± = variable inotropic response

arations to certain prostaglandins. One can only conclude that species, strain and tissue type are possible major determinants in the responsiveness of cardiac tissue to this important class of substances. Only extensive trials in man will answer the question of whether or not agents such as PGE_1 or PGE_2 have a significant inotropic effect in man. The in vitro data to date appear not to be consistent enough to confidently predict the effect in humans.

This kind of problem is further illustrated by the diversity of inotropic responses reported for glucagon in various test systems, including intact man (Table V). At the least, the data to date indicate that the canine preparations are exquisitely sensitive to the positive inotropic effects of glucagon. Man does not appear to be either consistent in his response, or quantitatively similar in reactivity. Undoubtedly the presence or absence of disease states and presumed associated biochemical derangement (e.g., adenyl cyclase) may influence the ultimate reactivity to this substance.

The many traps and pitfalls associated with the evaluation of hormones is further exemplified by the diversity of responses reported for the direct cardiac stimulant actions of aldosterone. Table VI summarizes some results obtained by various investigators. The fact that the use of any given preparation (e.g., cat papillary) yielded different results certainly suggests that other possible technical factors besides species, strain or tissue type are involved in determining the action of this steroid on the heart. The implication of these findings to human cardiac disease states or therapy remains to be determined.

TABLE V.—*Species Differences in Inotropic Responses to Glucagon*

Tissue	Species	Effect	Investigator**
Left Atrium	Human	0; ±	Levy
Papillary	Human	0	Prasad
L. Papillary	Human	±	Parmley et al.
L. Papillary	Human	+	Strauer
L. Papillary	Cat	+++	Parmley et al.
L. Papillary	Dog	++++	Parmley et al.
L. Vent.*	Sheep	0	Scholkens
L. Vent.*	Human	±	Various

* In vivo
** See References
+ = positive inotropism
0 = no significant effect
± = variable inotropic response

TABLE VI.—*Does Aldosterone Produce a Positive Inotropic Effect?*

Tissue/organ	Response	Investigator*
Rat Heart	+	Ballard et al.
Cat Papillary	+	Lefer et al.
Cat Papillary	+	Tanz
Monkey Papillary	+	Nayler
Rabbit L. Atria	0	Levy
Frog Vent.	0	Hajdu
Rat Papillary	0	Tanz
Rat Trab. Carneae	0	Ullrick
Rat Trab. Carneae	0	Tanz

* See References
+ = positive inotropism
0 = no effect

The fact that any given preparation may yield substantive differences in response to a given agent reinforces the need to understand the multitude of factors that can influence tissue responses in vitro. Table VII lists some of the better known and appreciated elements. The more we know about how any given test substance (e.g., norepinephrine) is influenced by the factors listed, the more complete and precise our knowledge will be on how and where it acts to produce its effects. The less we know about interactions of these factors with the test substance, the less confident we can be about the true biological effects on that given tissue or test system. Unfortunately for most agents, we are woefully ignorant of how these factors operate to influence the actions on the tissues we are studying.

ISOLATED HUMAN CARDIAC TISSUE

The use of human cardiac tissue for drug studies is not a recent phenomenon. What has

TABLE VII.—*Factors Influencing Response of Tissues in Vitro*

1. Spontaneous changes in sensitivity or responsiveness of tissue.
2. Desensitization, tachyphylaxis or tolerance as result of prior exposure to agent.
3. Net response based on resultant of actions of agent on several receptor sites in tissue.
4. Release of tissue mediators or factors (e.g., norepinephrine, acetylcholine).
5. Enzymatic degradation of agent.
6. Removal of agent from receptor site by active transport or uptake to other site.
7. Equilibration conditions (exposure time).
8. Removal of agent from medium (diffusion, absorption, uptake, degradation).

developed during the past 10–15 years, however, is directly attributable to the advent and extent of open heart surgical procedures in man. The widespread application of open heart surgery has added a new dimension for the investigator specializing in in vitro techniques. The reason is that he now is able to routinely and conveniently obtain freshly biopsied segments of the human heart which become available as the result of surgical interventions.

Table VIII summarizes a few of the types of human cardiac tissues employed in recent years for in vitro physiological, pharmacological and biochemical studies. Significantly, human cardiac tissues from human foetuses obtained from elective abortion procedures may add still another dimension to in vitro studies of human tissue.

Our own experience during the past 14 years has been mostly with the use of human right and left atrial tissue obtained upon commencement of total cardiopulmonary bypass procedures. We have used this tissue over preference to papillary muscle for several reasons. Some of these are listed in Table IX. These listed advantages of atrial tissue are applicable also to the selection of animal atrial preparations.

TABLE VIII.—*Examples of Human Heart Tissues Studied in Vitro*

Tissue	Investigator*
Left Atrium	Levy
	Sleator et al.
	van Dam & Durrer
Right Atrium	Levy
	Sleator et al.
	Noren et al.
	Ko & Paradise
Right Papillary (RV)	Chidsey et al.
	Prasad et al.
Trabeculae Carneae (RV)	Trautwein et al.
Papillary (human foetuses)	Gennser & Nilsson
Trabeculae muscle (post mort.)	Ullrick

* See References

TABLE IX.—*Some Advantages of Isolated Atrial Preparations*

1. Thinness permits good 0_2 diffusion.
2. Extensive tissue trimming or slicing unnecessary.
3. Uniformity of tissue size.
4. Adequate tension development.
5. Easy separation of right and left atria.
6. Size and shape eliminates need for ties and ligatures for mounting.
7. Stable contractile tension following equilibration.
8. Abundant adrenergic and cholinergic innervation.
9. Tissue size permits biochemical and chemical analyses without tissue pooling.

ISOLATED HUMAN SAPHENOUS VEINS

In 1964 we reported on the use of fresh, biopsied human veins for routine investigations in the pharmacology laboratory. During the intervening years, we have been able to expand our investigations to the use of both normal and varicosed vein tissue. Our most recent work has involved the use of saphenous vein segments obtained from patients undergoing aortocoronary vein bypass surgery.

We have utilized the helically cut vein strip preparation which was developed earlier for use with rabbit aortic strips.

While the human vein preparation has yielded results that are generally qualitatively and quantitatively similar to results obtained with animal arterial tissue, there are some important features of the vein which necessarily preclude applying or extrapolating results to other vascular tissues. Of course, other human vascular tissues can be obtained from a variety of surgical procedures. The use of other human vascular preparations for in vitro study has been reported.

SUMMARY

A survey has been made of the use, advantages and limitations of isolated animal and human myocardial and vascular tissue for in vitro investigations. Important qualitative and quantitative differences and similarities in human and animal tissues' responses to various substances are described.

The diversity of data suggest these important points: It is not wise to use and depend on one kind of in vitro preparation in characterizing the action of any given agent. Use of functionally deranged (natural or experimentally induced) animal tissues does not necessarily mimick the same disease or derangement in man, or predict responses to drugs in the intact organism.

REFERENCES

1. BALLARD, K., LEFER, A., and SAYERS, G. Effect of aldosterone and of plasma extracts on a rat heart-lung preparation. Am. J. Physiol. 199:221–225, 1960.
2. BARANCO, A., J. V. LEVY, R. ABBOTT, J. RUSSELL, J. SHORE, E. JOHNSON, and A. VUJEVICH. On-line computer control, testing and analysis of myocardial effects of chemical agents. Proc. Conf. Engineering in Med. and Biol., Washington, D. C. (1970), pp. 37–48.
3. BERTI, F., LENTATI, R., and USARDI, M. The species specificity of prostaglandin E_1 effects on the isolated heart. Med. Exptl. 13:233–240, 1965.
4. CHIDSEY, C., SONNENBLICK, E., MORROW, A., and BRAUNWALD, E. Norepinephrine stores and contractile force of papillary muscle from the failing human heart. Circulation 33:43–51, 1966.
5. GENNSER, G., and NILSSON, E. The relation between the action potential and the active state in human fetal myocardium and its dependence on muscle length and contraction frequency. Acta Physiol. Scand. 73:42–53, 1968.
6. HAJDU, S. Bioassay for cardiac active principles based on the staircase phenomenon of the frog heart. J. Pharmacol. 120:90–98, 1957.
7. KO, K-C, and PARADISE, R. The effects of substrates on halothane-depressed isolated human atria. Anesthesiology 33:508–514, 1970.
8. KOCH-WESER, J. Myocardial actions of angiotensin. Circulation Research 14:337–344, 1964.
9. KONES, R., and PHILLIPS, J. Glucagon: Present status in cardiovascular disease. Clin. Pharmacol. & Therap. 12:427–444, 1971.
10. LEFER, A., and SAYERS, G. Antagonism of the inotropic action of ouabain by aldosterone. Am. J. Physiol. 208:649–654, 1965.
11. LEVY, J. V. Force-frequency relationship of human atrial muscle in vitro: beta-adrenergic mechanism. Arch. Int. Physiol. et Biochim. 76:680–692, 1968.
12. LEVY, J. V., and KILLEBREW, E. Inotropic effects of prostaglandin E_2 on isolated cardiac tissue. Proc. Soc. Exp. Biol. & Med. 136:1227–1231, 1971.
13. LEVY, J. V. Myocardial and local anesthetic actions of B-adrenergic receptor blocking drugs: Relationship to physicochemical properties. Europ. J. Pharmacol. 2:250–257, 1968.
14. LEVY, J. V. Comparative actions of beta-adrenergic receptor blocking drugs on the contractile and electrical properties of isolated human atrial muscle. Cardiologia 53:293–305, 1968.
15. LEVY, J. V., and V. RICHARDS. Some contractile responses of isolated human vein strips. Clin. Res. 12: No. 1, 1964. (Abstract)
16. LEVY, J. V., and V. RICHARDS. Effect of aldosterone, ouabain and deoxycorticosterone on oxygen uptake of isolated cardiac muscle. J. Pharmacol. Exp. Therap. 144:104–109, 1964.
17. LEVY, J. V. Isolated Atrial Preparations, in *Methods in Pharmacology* (Ed. A. Schwartz) Vol. I, p. 77–104, Appleton-Century-Crofts, N.Y. 1971.
18. LEVY, J. V. Effects of papaverine, aminophylline, prostaglandins E_1 and E_2, and catecholamines on contractile responses of human and rabbit myocardium and vascular tissue. Proc. Intl. Conf. Physiology and Pharmacology of cyclic AMP, Milan, Italy, 1971.
19. LEVY, J. V. Inhibitory effects of antiinflammatory drugs on PGE_2-induced contractions of human vein strips in vitro. Clin. Res. 20:211, 1972.
20. LEVY, J. V. Unpublished data.
21. MANTEGAZZA, P., and PICCININI, F. (eds.). *Methods in drug evaluation.* North Holland Publ. Co., Amsterdam, 1966.
22. NOREN, G., GOLDRING, D., and SLEATOR, W. Tolerance of human and rat atrial muscle to hypothermia and anoxia. Circulation Res. 9:1229–1232, 1961.
23. NAYLER, W. G. The inotropic action of delta-aldosterone on papillary muscles isolated from monkeys. J. Pharmacol. 148:215–217, 1965.
24. NUTTER, D., and RATTS, T. Inotropic and chronotropic actions of prostaglandins E_1, A_1 and F_2alpha. Clin. Res. 20:29, 1972.
25. PARMLEY, W., CHUCK, L., and MATLOFF, J. Diminished responsiveness of the failing human myocardium to glucagon. Cardiology 55:211–217, 1970.
26. PRASAD, K., and CALLAGHAN, J. Effect of replacement of potassium by rubidium on the transmembrane action potential and contractility of human papillary muscle. Circulation Res. 24:157–166, 1969.
27. SCHOLKENS, B. Uber die Kardiostimulatorische und Antiarrhythmische Weskung von Sekretin und Glucagon. Acta Pharmacol. et Tox. 29:52, 1971.
28. SLEATOR, W., and DE GUBAREFF, T. Effects of calcium, acetyl choline and epinephrine on action potentials on human atrial muscle in vitro. Physiologist 3:144, 1960.
29. STRAUER, B. The influence of glucagon on myocardial mechanics of papillary muscles obtained from patients with chronic congestive heart failure. Arch. Pharmak. 270:90–93, 1971.
30. TANZ, R. Studies on the inotropic action of aldosterone on isolated cardiac tissue preparations; including the effects of pH, ouabain and SC-8109. J. Pharmacol. 135:71–78, 1962.
31. TRAUTWEIN, W., KASSEBAUM, D., NELSON, R., and HECHT, H. Electrophysiological study of human heart muscle. Circulation Res. 10:306–312, 1962.
32. ULLRICK, W., LENTINI, E., and SOMMERS, S. Summation and tetanus in postmortem human heart muscle. J. Appl. Physiol. 14:567–568, 1959.
33. ULLRICK, W., and HAZELWOOD, R. Influence of aldosterone on isometric tension development in the rat heart. Am. J. Physiol. 204:1001–1004, 1963.
34. VAN DAM, R., and DURRER, D. Excitability and electrical activity of human myocardial strips from the left atrial appendage in cases of rheumatic mitral stenosis. Circulation Res. 9:509–514, 1961.

DISCUSSION

CHAIRMAN COHEN: Dr. Levy, if you had your choice, as you do in many cases, to pick out one preparation that you felt was most likely to give you some meaningful answers in terms of the pharmacology of a new agent, would you use your isolated atrial muscle, papillary muscle, or an intact preparation?

DR. LEVY: Basically as an in vitro pharmacologist, if I can use that phrase, I would choose the rabbit left atrium and the cat papillary as the most representative. I think we would cover most of the area using those two preparations. Of course, as I showed on the earlier slide, one often may be surprised, despite what you see on these isolated preparations, when one puts the same material into the intact animal or in man. I would like to think that the earlier the new substance can be tried in man, the less likely it is that we are going to get into trouble and deal with false positives. But I prefer the rabbit left atrium and the cat papillary as major predictors of myocardial effects.

SIDNEY CASSIN, University of Florida, Gainsville: I'm just curious about the relationship and the choice of agonist and antagonist that you use. I'm curious whether you would comment about an interaction between aldosterone, propranolol and ouabain? Do you use these in agonist-antagonist experiments?

DR. LEVY: In this particular case we were testing the question, "Does aldosterone antagonize the cardiotonic effect of ouabain?" That was one question being tested. If you pretreated the human or rabbit atrium with the mineralo-corticoid, then challenged with the glycoside, you did not block the normal inotropic response.

DR. CASSIN: What reason did you have to believe that you might get a response?

DR. LEVY: In antagonism?

DR. CASSIN: Yes.

DR. LEVY: According to the Wilbrandt hypothesis, the mineralo-corticoid was a competitive antagonist of ouabain at the membrane level in terms of sodium transport. This was a very early hypothesis that was tested on red blood cells. In that particular system, in terms of ionic transport, there was a competition. Yet on the contractile system of the atrium of the rabbit and on the human, there was no antagonism.

DR. CASSIN: And the propranolol. . . .

DR. LEVY: And similarly, with propranolol. We tested whether the inotropic effect of ouabain was mediated through a catecholamine mechanism or a beta adrenergic mechanism by using it on the rabbit atrium. We first gave the antagonist, then gave the glycoside, which produced a normal effect. When we tested it on the human atrium, propranolol was found not to antagonize the cardiotonic effect of the glycoside.

A STUDY OF CELL KINETICS IN THE RAT THYMUS AFTER PERTURBATION BY DEXAMETHASONE

J. C. Pierce*

Cell kinetics were studied in the normal rat thymus and after the intravenous injection of 0.2 mg. of dexamethasone. This caused a decrease in DNA-P content from 910 μgm to 88 μgm 3 days after injection with a return to 798 μgm at 10 days. The hourly fractional rate of DNA turnover estimated from the time specific activity curves of DNA-P and acid soluble phosphorus after the intraperitoneal injection of ^{32}P decreased from a control level of 0.040/hr. to a minimum of 0.004/hr., 24 hours after dexamethasone. Cell production in the thymus was reduced from 54 x 10^6/hr. in the normal thymus to 1.8 x 10^6 at 24 hours after dexamethasone and returned to normal at 10 days. Endogenous cell production was more than sufficient to account for the observed increment in DNA-P during regeneration of the thymus.

The data suggest that cells are produced by a stem cell population which constitutes 20% of the thymus and which divides repeatedly with a turnover time of approximately 5 hours. One-half of the daughter cells are retained within the stem cell population. Of the remaining newly formed cells, approximately three quarters are probably destroyed in the thymus soon after mitosis is completed. The remaining cells enter the small thymocyte compartment where differentiation occurs. It was estimated that these cells are retained for 48–72 hours before they are lost from the thymus to secondary lymphoid organs. The rate of loss of DNA from the thymus and the profound decrease in the rate of cell production which followed a single dose of dexamethasone suggested that all of the cells in the replicating compartment and 20% of the cells in the small thymocyte compartment were exquisitely sensitive to killing by dexamethasone, but with the passage of time after mitosis, the small thymocytes differentiated and lost this sensitivity.

INTRODUCTION

The thymectomy experiments of Good and co-workers[1] in neonatal animals have indicated the central role which the thymus plays in the development of immunological capacity. The experiments of Metcalf and his co-workers have suggested that the thymus in the adult continues to produce very large numbers of cells,[2,3] and that the presence of the thymus still plays an important role in sustaining the immunological capacity of the animal.[4] The present experiments were undertaken to study the kinetics of thymus cell production and loss from the adult thymus during the major changes in the rate of cell production caused by dexamethasone which is a synthetic adrenal corticosteroid with great anti-inflammatory potency and no sodium retaining effect.

METHODS

Adult female Sprague-Dawley rats which weighed approximately 200 gms. were used. The rats were obtained from Charles River and acclimated in an air conditioned room for at least one week prior to the first experimental manipulation. Dexamethasone (9 α-fluoro-16 α-methyl-prednisolone) was given as a single dose of 0.2 mg. intravenously via a tail vein at various intervals prior to sacrifice. The deoxyribonucleic acid (DNA) content of the thymus was estimated by the diphenylamine method of Burton.[5] DNA was used as a standard and results are reported as DNA phosphorus (DNA-P). The hourly fractional turnover rate for DNA was estimated from the time specific activity curves of phosphorus in DNA and in the acid soluble phosphorus pool at one and two hours after the intraperitoneal injection of one μc of ^{32}P per gram body weight as has been previously described.[6] The rate of cell production was calculated by multiplying the DNA-P content of the thymus times the DNA hourly turnover rate and dividing by the average DNA-P content of a rat cell, 0.678 x 10^{-12} gms.[7]

* Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia.

Autoradiographs were prepared from thymus imprints on gelatin coated slides which were fixed in ten percent formalin with phosphate buffer. They were dipped in Kodak NTB-3 nuclear track emulsion, exposed for eight weeks at 4° C and developed with Kodak D-19 developer and F-5 fixer at 18° C. For autoradiographic studies, the rats were injected with five μc per gram body weight of ^{3}H-deoxycytidine intraperitoneally one hour prior to sacrifice. Routine histological sections of the thymus fixed in phosphate buffered ten percent formalin and stained with hematoxylin and eosin were examined at zero and eight hours and at one, two, three, four, five, seven, and ten days after dexamethasone injection.

RESULTS

The rate of DNA synthesis in the thymus reached its minimum one day after dexamethasone while the DNA content of the thymus reached its minimum at three days and the rate of cell production was reduced to a minimum during the entire interval one to three days after dexamethasone (Table I). By ten days, these three parameters had returned essentially to normal. The percentage of cells labeled with ^{3}H-deoxycytidine in autoradiographs paralleled the changes in DNA turnover. One to three days after dexamethasone, the percentage of labelled cells fell to the range of five to ten percent as compared with twenty percent in untreated controls. Five days after dexamethasone, fifty to sixty-five percent of the cells were synthesizing DNA and by ten days this had returned essentially back to normal.

Histologically there were massive numbers of pycnotic thymic lymphocytes in the cortex eight hours after dexamethasone. At one day, the cortex was reduced in width and the numbers of these pycnotic nuclei had diminished. At three days, the thinned cortex consisted chiefly of epithelial cells while the medulla contained many lymphocytes, a reversal of the cortico-medullary ratio. At five days in the cortex there was a significant return of thymic lymphocytes, many of which were undergoing mitosis and by ten days, the histological appearance had been nearly restored to normal.

TABLE I.—*Response of the Adult Rat Thymus to a Single Intravenous Injection of Dexamethasone*

Time after dexamethasone	DNA-P content μgm	DNA turnover per hour	Cell production per hour
0	910	0.040	54×10^6
1 day	352	0.0035	1.8×10^6
3 days	88	0.056	7.3×10^6
5 days	266	0.082	32×10^6
10 days	798	0.045	53×10^6

DISCUSSION

The thymus may be divided into three compartments: A replicating cell compartment, a differentiating cell compartment, and a residual cell compartment. The replicating cell compartment consists of stem cells that divide repeatedly. Approximately one-half of the daughter cells are retained in this compartment as stem cells and of the remaining newly formed cells, a portion of them migrate from the thymus or are destroyed within the thymus soon after mitosis is completed. The remaining daughter cells enter the second compartment which consists chiefly of small thymocytes where they remain for a period of time and where differentiation of some of the cell membrane determinants occurs. The third or residual cell compartment consists of epithelial and stromal cells and medullary lymphcytes which are long-lived and unaffected by adrenocorticosteroids.

The size of the replicating compartment can be estimated from the percentage of cells labeled at one hour on autoradiographs with ^{3}H-deoxycytidine to contain about twenty percent of the thymus cells. The residual cell compartment can be estimated from the fraction of cells remaining when the thymus attains its minimum size after dexamethasone to contain ten percent of the thymus cells. The differentiating or small thymocyte compartment contains the balance of thymus cells or seventy percent of the thymus. The average generation time of cells in the replicating compartment was approximately five hours on the basis of the hourly fractional turnover rate for the whole thymus and the size of the replicating compartment. The average holding time for cells in the small thymocyte compartment can be estimated

at 48 to 72 hours from the time between the reduction of DNA synthesis to its minimum and the reduction of DNA content to its minimum value. This is consistent with the value that Weissman[8] has observed for the lag time between local labeling of thymocytes and their maximum time of migration to the periphery. If 72 hours is the average holding time for thymocytes in the differentiating compartment, it can be calculated that this compartment accommodates one-fourth of the net cell production of the replicating compartment. This implies that three-quarters of the cells which were newly formed in the replicating compartment either migrated from the thymus or were destroyed within the thymus very soon after mitotic division was completed.

Because of the lag between the time that the DNA of cells was labeled in the thymus and the appearance of these cells in peripheral tissues,[8] most of these cells probably died in the thymus. The sensitivity of the replicating cells in the thymus to dexamethasone was remarkable. The rate of cell production which gave a more accurate measure of the size of the replicating pool than either the DNA turnover rate or the DNA content of the thymus was reduced to three percent of the control value one day after dexamethasone. The rate of DNA loss from the thymus during the initial 24 hours, however, could not be accounted for solely by loss of the replicating cells and normal migration from the differentiating or small thymocyte compartment. An additional amount of DNA equal to approximately twenty percent of the cells in the differentiating compartment was lost during this period. During the next two days the rate of DNA loss from the thymus was consistent with the normal rate of loss of cells from the differentiating compartment. This suggests that while thymocytes during division and immediately after were exquisitely sensitive to dexamethasone during the additional time that these cells resided in the thymus prior to migration, this sensitivity to dexamethasone was lost. This loss of sensitivity to dexamethasone appears to be similar to the loss of alloantigens which has been observed during differentiation of thymus cells.[9]

SUMMARY

Thymus cell kinetics were studied in normal rats and after treatment with 0.2 mg. of dexamethasone, intravenously.

The studies are consistent with a thymus model with three compartments: a replicating cell compartment, a differentiating cell compartment, and a static cell compartment.

It was concluded that all of the cells in the replicating cell compartment and twenty percent of the cells in the differentiating cell compartment are exquisitely sensitive to dexamethasone but that thymocytes lose this sensitivity as they undergo further differentiation in the differentiating compartment.

REFERENCES

1. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., and Papermaster, B. W. The role of the thymus in development of immunologic capacity in rabbits and mice. J. Exp. Med. 116:773–796, 1962.
2. Metcalf, D., and Wiadrowsky, M. Autoradiographic analysis of lymphocyte proliferation in the thymus and in thymic lymphoma tissue. Cancer Res. 26:483–491, 1966.
3. Matsuyama, M., Wiadrowski, M., and Metcalf, D. Autoradiographic analysis of lymphopoiesis and lymphocyte migration in mice bearing multiple thymus grafts. J. Exp. Med. 123:559–576, 1966.
4. Metcalf, D. Delayed effect of thymectomy in adult life on immunological competence. Nature 208:1336, 1965.
5. Burton, K. A. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315–323, 1956.
6. Pierce, J. C. A model of cell production in the lymph nodes of normal and allografted rats. Lab. Invest. 19:494–502, 1968.
7. Thomson, R. Y., Heagy, F. C., Hutchison, W. C., and Davidson, J. N. The deoxyribonucleic acid content of the rat cell nucleus and its use in expressing the results of tissue analysis, with particular reference to the composition of liver tissue. Biochem. J. 53:460–474, 1953.
8. Weissman, I. Thymus cell migration. J. Exp. Med. 126:291–304, 1967.
9. Owen, J. J. T., and Raff, M. C. Studies on the differentiation of thymus-derived lymphocytes. J. Exp. Med. 132:1216–1232, 1970.

APPLICATION OF MINIATURE SWINE TO ANESTHETIC STUDIES ON THE CARDIOVASCULAR SYSTEM AND HEPATIC INHALATION ANESTHETIC METABOLISM

D. C. Sawyer*, E. I. Eger, II** and W. V. Lumb***

Miniature swine were used for studies on the cardiovascular effects of anesthetics and hepatic metabolism of inhalation anesthetics. Hormel swine were selected to study effects of halothane, methoxyflurane, pentobarbital and thiamylal on cardiac output, stroke volume, mean arterial pressure, heart rate and related variables. The experimental design was to study each anesthetic at 2 week intervals. This provided a maximum of 48 periods for 12 pigs. Thirty-eight of 48 runs were accomplished. Incomplete periods in the design resulted from aortic rupture subsequent to flow sensor implantation in 3 animals and nonfunctional aortic catheters in 3 animals. Duration of instrumentation averaged 60.6 (25–127) days. Representative and consistent control data were obtained and compared with data recorded during and following anesthesia. Each anesthetic decreased cardiac output and stroke volume. Heart rate, mean arterial pressure and peripheral vascular resistance were variably affected. For metabolism studies, catheters were implanted in the aorta, portal vein and common hepatic vein of miniature pigs. A common hepatic vein was formed to isolate the liver for *in vivo* metabolic studies. Animals were used 34–150 days postimplantation. These studies demonstrated that the liver extracts (metabolizes) a greater fraction of halothane and fluroxene from hepatic blood at lower than at higher anesthetic partial pressures. From exposure to low subanesthetic concentrations for periods of 20 hours to 1 week, considerable hepatic metabolism of halothane, methoxyflurane and fluroxene was found with little or no metabolism of Ethrane and no metabolism of cyclopropane, nitrous oxide or Forane.

INTRODUCTION

When animals are selected as subjects for study, 2 major considerations must be made. First, from previous investigations, which animal would be the most desirable from a physiological or pharmacological point of view? Second, which animal would be most conducive to allow accomplishment of the study? For the investigations described in this paper regarding the effects of anesthetics, miniature swine were selected as research subjects because physiological responses are similar to those found in man and other animals. In addition, swine readily adapt to the laboratory environment and can be used for chronic instrumentation studies.

Miniature swine were used to study cardiovascular effects of halothane, methoxyflurane, pentobarbital and thiamylal.† In a separate study, miniature swine were selected to investigate hepatic metabolism of inhalation anesthetics.††

METHODS

Twelve Hormel miniature swine, 30–35 kg were surgically instrumented to study cardiovascular effects of anesthetics.[1] During general anesthesia with halothane,[2] a left thoracotomy was performed.[3] An electromagnetic flow sensor was placed around the ascending aorta. Silicone rubber catheters were placed in the thoracic descending aorta and superior vena cava.

After a 10-day recovery period, control measurements were made from unmedicated animals at 2-day intervals and immediately prior to

* Associate Professor, Department of Small Animal Surgery & Medicine, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48823.

** Professor, Department of Anesthesia, University of California, San Francisco, California 94122.

*** Professor and Director, Surgical Laboratory, Colorado State University, Fort Collins, Colorado 80521.

† This work was conducted at the Surgical Laboratory by Dr. Sawyer and Dr. Lumb and was supported by U.S. Public Health Service Grant GM-HE-14456-02 and Special Fellowship Award 5-F2-GM-29, 932-02.

†† Investigations were conducted at the San Francisco Medical Center by Dr. Sawyer, Dr. Eger and others and was supported by U.S. Public Health Service Grants 5T 1 GM-0063-12, 1 PO 1 GM-15571-01A1 and 2 FO3-FM-29932-03, the Ayerst Laboratories, and Ohio Medical Products, a Division of Air Reduction Company, Inc.

each anesthetic period. Arterial blood samples were obtained to establish control blood gas values.

For 2 weeks prior to surgical instrumentation, animals were brought to the laboratory from their outside pens and placed on a table for 10–20 minutes. This procedure allowed them to adapt to the laboratory environment and personnel. During the postoperative period, the same procedure was followed. As a result, when control recordings of cardiovascular variables were made, animals rested quietly on the table.

Four anesthetics were used in this study: halothane, methoxyflurane, pentobarbital sodium and thiamylal sodium. The experimental design was to administer each of the 4 anesthetics at 2 week intervals to 12 animals. Ideally, this would result in 48 anesthetic periods. During each period, anesthesia was induced with the drug to be studied and a 1 hour equilibration period followed. Spontaneous ventilation was assisted by manual compression of the rebreathing bag. The following 2 hours were used to determine the effects of each anesthetic on cardiac output and related variables. Assisted spontaneous ventilation was provided during study periods.

Administration of an anesthetic was discontinued 3 hours after induction. Variables were recorded at 15 minute intervals during anesthesia and 1, 2, 3, 24, 96 and 144 hours postanesthesia.

For metabolism studies, Hormel swine, 35–45 kg, were selected.[4] These animals were conditioned to the laboratory environment as described previously. During surgical anesthesia with halothane, nylon intravascular catheters were implanted to permit later sampling of blood from the aorta, portal vein and common hepatic vein following recovery from surgery. Silastic tubing was secured to one end of the nylon catheter. The portal vein was cannulated with the Silastic portion via the splenic vein. A common hepatic vein was constructed using a modification of the method of Vetto and Boge.[5] A 2-cm section of Tygon tubing with catheter attached was implanted in the inferior vena cava 1 cm anterior to the renal veins. The slow formation of a clot in this tubing isolated the upper inferior vena cava, creating a common hepatic vein. The slow clotting permitted the development of collateral venous pathways posterior to the tubing via the vertebral and azygos veins. Between the right atrium and the occluded tubing, blood entering the vena cava flowed from the major and minor hepatic veins, since the phrenic veins had been ligated during the surgical procedure. The catheter tip attached to the Tygon tubing was placed at the level of the major hepatic veins. Catheters were implanted in the abdominal vena cava and aorta via the femoral vein and artery. During the postoperative period, indocyanine green dye was injected through the femoral-vein catheter and blood was sampled from the common hepatic vein. When no dye was immediately detected in blood from the hepatic vein, we concluded that complete thrombosis of the caval tubing had occurred. Thrombosis occurred 26–62 days postimplantation.

Studies were conducted after occlusion of the caval tubing. Halothane was administered to induce anesthesia and measurements were made after alveolar concentrations had been held constant for 45–60 minutes at 3 different levels.[4] Thereafter, control of alveolar concentrations were discontinued. Blood samples from the portal vein, aorta and common hepatic vein were obtained simultaneously from unmedicated animals. The volume of anesthetic in hepatic venous blood divided by the total inflow volume yields the minimum fraction removed by the liver, and this fraction was defined as anesthetic metabolized.

In a similar study,[6] animals were exposed only to subanesthetic concentrations to demonstrate the extent of hepatic metabolism of halothane, methoxyflurane, cyclopropane, Ethrane††† (2-chloro-1, 1, 2-trifluoroethyl difluoromethyl ether), Forane††† (1-chloro-2, 2, 2-trifluoroethyl difluoromethyl ether) and nitrous oxide. After establishment of equilibrium, fraction of anesthetic removed by the liver was determined.

RESULTS

Data obtained from cardiovascular studies are presented (Tables I–IV).[1] After halothane

††† Trademark, Ohio Medical Products, Division of Air Reduction Company, Inc.

TABLE I.—*Cardiovascular and Blood Values Record From Nine Miniature Swine (32 ±4 kg) Before and During Halothane Anesthesia*

	Preanesthesia control values	Anesthesia	% change
CO (ml/min)	3244 ± 668	1600 ± 235	−50
SV (ml)	28 ± 6	14 ± 2	−50
Stroke Work (mm Hg .1)	2.91± 1.06	0.89± .24	−69
MAP (mm Hg)	106 ± 30	62 ± 8	−41
HR (beats/min)	118 ± 18	119 ± 17	0
PVR (mm Hg/1/min)	36 ± 9	41 ± 9	+14
Hct. (%)	40 ± 3	31 ± 3	−22
pH	7.49± .02	7.43± .03	
P_{aCO_2} (mm Hg)	34.2 ± 3.3	46.5 ± 5.8	
Bld. anes. conc. (mg/100 ml)	0	20.1 ± 1.6	
Calc. alv. conc. (%)	0	1.1	

± = Standard deviation

anesthesia, variables returned to control levels in 2 hours. Pigs were usually able to stand within 1 hour and eat within 3 hours. After methoxyflurane anesthesia, variables did not return to control levels for 24–96 hours postanesthesia and animals appeared mildly depressed for 24 hours. After pentobarbital anesthesia,

TABLE II.—*Cardiovascular and Blood Values Recorded From Ten Miniature Swine (32.7 ± 3 kg) Before and During Methoxyflurane Anesthesia*

	Preanesthesia control values	Anesthesia	% change
CO (ml/min)	3083 ± 600	1898 ± 678	−39
SV (ml)	27 ± 4	13 ± 3	−48
Stroke Work (mm Hg .2)	3.09± 0.54	1.05± 0.30	−66
MAP (mm Hg)	114 ± 15	82 ± 10	−29
HR (beats/min)	113 ± 18	140 ± 21	+20
PVR (mm Hg/1/min)	39 ± 9	49 ± 15	+21
Hct. (%)	42 ± .04	36 ± 3	
pH	7.46± .04	7.42± .05	
P_{aCO_2} (mm Hg)	35.1 ± 2.8	51.4 ± 4.5	
Bld. anes. conc. (mg/100 ml)	0	18.6 ± 0.7	
Calc. alv. conc. (%)	0	0.22	

± = Standard deviation

TABLE III.—*Cardiovascular and Blood Values Recorded From Nine Miniature Swine (32.7 ± 3 kg) Before and During Thiamylal Anesthesia*

	Preanesthesia control values	Anesthesia	% change
CO (ml/min)	3022 ± 686	1900 ± 466	−38
SV (ml)	26 ± 5	15 ± 5	−41
Stroke Work (mm Hg .1)	3.02± .75	2.42± .94	−20
MAP (mm Hg)	114 ± 13	154 ± 20	+34
HR (beats/min)	115 ± 17	127 ± 28	+ 9
PVR (mm Hg/1/min)	40 ± 10	87 ± 25	+122
Hct. (%)	39 ± 4	33 ± 4	−16
pH	7.48± .03	7.45± .06	
P_{aCO_2} (mm Hg)	34 ± 3	41 ± 6	
Bld. anes. conc. (mg/100 ml)	0	3.6 ± 0.5	

± = Standard deviation

variables did not return to control levels for 96–144 hours. Pigs became ambulatory 5 hours after anesthetic administration had ceased and were ataxic for over 24 hours. The same was true after thiamylal anesthesia, but even 24 hours postanesthesia, animals were reluctant to stand and would not eat for 36–48 hours.

Detailed results of anesthetic metabolism have been published.[4,6,7] From investigations following halothane (Table V)[7] and fluroxene

TABLE IV.—*Cardiovascular and Blood Values Recorded From Ten Miniature Swine (32.7 ± 3 kg) Before and During Pentobarbital Anesthesia*

	Preanesthesia control values	Anesthesia	% change
CO (ml/min)	3237 ± 675	1914 ± 367	−41
SV (ml)	28 ± 4	16 ± 3	−45
Stroke Work (mm Hg .1)	3.32± .77	1.99± .52	−40
MAP (mm Hg)	119 ± 21	128 ± 17	+8
HR (beats/min)	117 ± 18	123 ± 8	+8
PVR (mm Hg/1/min)	38 ± 9	70 ± 16	+86
Hct. (%)	40 ± 3	33 ± 4	−17
pH	7.50± 0.03	7.38± .06	
P_{aCO_2} (mm Hg)	33 ± 3	52 ± 11	
Bld. anes. conc. (mg/100 ml)	0	4.2 ± 0.9	

± = Standard deviation

TABLE V.—*Results of Hepatic Halothane Metabolism in in Miniature Swine*

Alveolar halothane (%)	Fraction halothane removed	Metabolic rate (ml/min)	Number Animals
1.6 ± .05	—.08 ± .07		3
1.1 ± .04	.05 ± .03		6
0.6 ± .03	.04 ± .04	.30 ± .37	7
0.25 ± .02	.13 ± .03	.57 ± .16	7
0.12, 0.09	.03, .32	.10, .56	2*
0.05 ± .006	.24 ± .05	.13 ± .05	3
0.01 ± .002	.34 ± .05	.07 ± .004	4
0.007 ± .006	.59 ± .04	.05 ± .006	4
0.0026 ± .0003	.77 ± .03	.04 ± .009	6
0.0006, .0004	.84, .85	.02, .005	2*

± = SE

* Individual values are presented.

anesthesia (Table VI),[7] anesthetic removed by the liver at or above anesthetic levels was not significant. However, as alveolar concentrations decreased during the postanesthetic period, fraction removed increased to almost complete extraction.

Results of the studies during which animals were exposed only to subanesthetic levels demonstrated that halothane and methoxyflurane are metabolized. There was little or no metabolism of Ethrane, and no detectable metabolism of cyclopropane, Forane or nitrous oxide.

DISCUSSION

To complete the cardiovascular studies, 63 days were required from the date of surgery through the week after the last anesthetic period. A functional flow sensor, a patent aortic catheter, and a healthy animal were required to meet this objective. The actual duration of instrumentation averaged 60.6 days with a range of 25–127 days. Instrumentation permitted measurement of consistent control data in unanesthetized and unmedicated animals which then could be compared with those data recorded during anesthesia and postanesthesia.

TABLE VI.—*Results of Hepatic Fluroxene Metabolism in One Animal*

Alveolar fluroxene (%)	Fraction removed
2.18	—.09
0.36	—.09
0.12	.42
0.062	.42
0.029	.74
0.0098	.85

As indicated, there were 48 possible anesthetic study periods. Thirty-eight of the 48 runs were accomplished. Six pigs had all 4, 3 had 3, 2 had 2 and 1 had only 1 evaluation. Incomplete periods resulted from aortic rupture subsequent to flow sensor implantation in 3 animals and nonpatent aortic catheters in 3 animals.

This instrumentation allowed beat-by-beat measurements of cardiovascular variables without other drug influences. As a result, representative and consistent control data were obtained for extended periods. Changes recorded during anesthesia were compared to control data and provided an excellent means for analyzing anesthetic effects on cardiac output and related variables. Each anesthetic decreased cardiac output, stroke volume, and stroke work. Hematocrit was consistently decreased. Heart rate, mean arterial pressure, and peripheral vascular resistence were variably affected.

Miniature swine are comparatively resistent to rupture of the ascending aorta following flow sensor implantation and are excellent animal subjects for examining anesthetic effects on the cardiovascular system.

For metabolism studies, miniature pigs were surgically implanted as described. Anesthetic management during vena cava occlusion was difficult as the procedure resulted in severe hypotension. A few animals died during surgery at the beginning of the study presumably due to severe hypotension. In subsequent animals, blood and fluids were administered intravenously during occlusion of the inferior vena cava to maintain arterial pressure near normal. This procedure decreased morbidity and mortality considerably.

Nine animals were instrumented and the duration of functional instrumentation averaged 78 (range 34–150) days. Time required for thrombosis of the caval tubing averaged 36 (range 26–62) days. The portal vein catheter was usually the first to become nonfunctional. The average time for blood sampling from the portal vein was 45 days with a range of 10–105 days. The number of times animals were studied ranged 1–5 with a mean of 3. It was difficult to achieve the complete objectives of this instrumentation as the portal vein catheter would not

allow withdrawal of blood not long after the caval tubing occluded.

Extensive adhesions precluded replacement of the portal vein catheter. However, repeated studies were possible in animals with functional catheters. Also, this instrumentation allowed studies to be conducted continuously over a period of days.

Results of this study indicate that uptake and distribution studies at low anesthetic concentrations cannot necessarily be used to predict events at concentrations used clinically. Also, metabolic studies of subanesthetic concentrations cannot be extrapolated with all anesthetics to predict metabolism during anesthesia. Because of their exposure to low concentrations of anesthetics which are metabolized, anesthetists may produce large amounts of metabolites relative to those produced by patients.

SUMMARY

Miniature swine appear to adapt well to relatively long term bioinstrumentation procedures. Pigs have restricted movement of their head and feet and cannot damage external catheters secured by bandages. When housed in the same or adjacent pens, they will cooperate to remove bandages. Therefore implanted animals must be separated. To prevent rubbing of catheters implanted dorsal and anterior to the shoulders, low overhangs of pen structures must not be available. An important factor is preconditioning to the laboratory environment before surgery and the minimal use of physical restraint. Animals, when brought to the laboratory from pens, were trained to walk to and from the table on a ramp. Pigs do not like to be picked up and scream quite vigorously before being placed on a table. This procedure is quite helpful when working with animals above 30 kg. Also, by avoiding excitement, consistent control data can be obtained from instrumented animals.

REFERENCES

1. Sawyer, D. C., Lumb, W. V., and Stone, H. L. Cardiovascular effects of halothane, methoxyflurane, pentobarbital, and thiamylal. J. Appl. Physiol. 30:36–43, 1971.
2. Sawyer, D. C., and Lumb, W. V. Halothane anesthesia in miniature swine. Anesthesia Analgesia Current Res. 49:616–619, 1970.
3. Sawyer, D. C., and Stone, H. L. Thoracic surgery for electromagnetic flow sensor implantation on miniature swine. In: *Swine in Biomedical Research*, edited by L. K. Bustad and M. P. Burns. Richland, Wash.: Atomic Energy Commission and Pacific Northwest Laboratory, 1966, p. 405–409.
4. Sawyer, D. C., Eger, E. I., II, Bahlman, S. H., Cullen, B. F., and Impelman, D. M. Concentration dependence of hepatic halothane metabolism. Anesthesiology 34:230–235, 1971.
5. Vetto, R. M., and Boge, C. H. A simplified method for constructing a common hepatic vein in the dog. Ann. Surg. 155:543–546, 1962.
6. Halsey, M. J., Sawyer, D. C., Eger, E. I., II, Bahlman, S. H., and Impelman, D. M. Hepatic metabolism of halothane, methoxyflurane, cyclopropane, Ethrane, and Forane in miniature swine. Anesthesiology 35:43–47, 1971.
7. Sawyer, D. C., Eger, E. I., II, Bahlman, S. H. Halsey, M. J., Cullen, B. F., and Impelman, D. M. Metabolism of inhalation anesthetics. In: *Cellular Toxicity of Anesthetics*, edited by B. Raymond Fink, the Williams and Wilkins Co., Baltimore, 1972.

DISCUSSION

Ed Jankus, University of Minnesota, St. Paul: I thank you for a very fine paper. I'd like to ask you a question concerning methoxyflurane particularly. Would you comment on the degree of analgesia achieved with that anesthetic in the pig in your experience, and would you also state the degree of acidosis that resulted and the mechanism that produced this?

Dr. Sawyer: We never evaluated the analgesia in these animals, so I really cannot comment. We were never stimulating them after anesthesia in the study. We used halothane to do the implants. So for that reason I really don't know. We were supposed to have some analgesia post-anesthesia and I think that it probably occurred, because the concentration of anesthetic is still around for a long time. As far as the acidosis, I think there is both a metabolic and a respiratory acidosis, as the PCO2 is elevated.

Dr. Jankus: How profound was that in your experiment?

Dr. Sawyer: Not terribly bad. We assisted ventilation. Animals were breathing spontaneously, but we assisted them as well. We just

didn't let them drift. We somewhat controlled the PCO2. But we didn't have an entire sample.

DR. JANKUS: Thank you.

GERALDINE KENT, Hospital for Sick Children, Toronto: You mentioned that you had a peripheral vascular constriction with halothane.

DR. SAWYER: Yes, a little bit. Only 14%.

DR. KENT: Right. Now the reason we use halothane for open heart surgery is to get a good peripheral vascular dilation. I suggest that the acidosis from inadequate ventilation produced your peripheral vasoconstriction and the Bird respirator isn't adequate for the pig.

DR. SAWYER: Well, the pig triggered the machine. And it was spontaneously breathing and I won't argue with you on that point.

DR. KENT: Well, are you then stating that halothane is a peripheral vasoconstrictor in the pig?

DR. SAWYER: Under the conditions of the study, under spontaneous ventilation, spontaneous assisted ventilation, it was.

DR. KENT: But halothane is a marked respiratory depressant in the pig.

DR. SAWYER: Right.

DR. KENT: And unless you're on top of that ventilation, you're losing out on your acidosis. Right? I suggest that you buy a volume cycle ventilator instead of a Byrd.

DR. SAWYER: I think that that's a very good point. Halothane has been noted as a vasodilator in many, many studies.

ALLEN INGLING, University of Maryland, College Park, Md.: I'd like to know two things: one, what was the material, and size, of the tubing you used in the portal vein? Secondly, what procedures did you use daily or every six hours to keep that material open?

DR. SAWYER: We used nylon. In the March issue of "Anaesthesiology," we have noted the size of the catheters and the other details of the study. We filled these catheters with heparin and then would flush these catheters every other day or from Friday to Monday, so there would be anywhere from two to four days between flushings.

ALDEN E. STILSON, Ohio State University, Columbus: I might mention the possible use of nitrous oxide together with halothane. I'm sure you have already used that in other cases. It might tend to ameliorate some of the wide fluctuations due to halothane by itself, by the simple process of reducing the amount of halothane necessary.

DR. SAWYER: Right. I agree with that.

HUGH CALDERWOOD, University of Florida, Gainsville: How do you determine surgical anaesthesia for a skin incision in a paralyzed animal?

DR. SAWYER: This animal was paralyzed only for about three minutes. And we had one hour before we evaluated the depth of anaesthesia, before we did the study. So we had one hour of equilibration and we assume that most of the paralyzing anaesthesia was dissipated by then. Spontaneous respiration resumed in about three minutes.

DR. CALDERWOOD: Using these animals with repeated different drugs, how do you think these drugs effect enzyme induction and metabolism of subsequent drugs?

DR. SAWYER: We may have seen that influence, I agree. We evaluated the control values between each study and found there was no significant difference between the controls prior to each of the four different anesthetics. There may have been some enzyme induction but we didn't study it.

QUESTIONER (Unidentified): Dr. Sawyer, Dr. Clarkson at our institution has come out very strongly for a clinical syndrome of halothane hepatic toxicity and he feels that this is a predictable response in perhaps 1 to 2% of the individuals who were repeatedly exposed to halothane. Did you make any observations in your swine who did receive halothane several times as to hepatic toxicity or unexplained fevers after exposure to it?

DR. SAWYER: I was looking for this, with great interest, and never found it. And I never saw any problem with it in pigs. I have only seen it in one dog clinically. And I'm looking for it with great fervor.

CHAPTER IX

ANATOMY AND PATHOLOGY

A Correlative Study of the Anatomy of the Cardiovascular Systems of Animals and Man

Direct Visualization of the Coronary Microcirculation of the Left Atrium of the Cat

Cardiac Effects of Hemorrhagic Shock in Dogs and Other Animals

Pathological Techniques Applied to Artificial Organ Research. An Animal Model for the Detection of Surface-Induced Thrombosis and Embolism

Spontaneous Cardiopulmonary Disease in Nonhuman Primates: Potential Models

Arteriosclerosis and Other Vascular Diseases in Zoo and Laboratory Animals

Alterations in DNA Synthesis and Other Metabolic Processes in Aortas of Cholesterol-Fed Swine

A CORRELATIVE STUDY OF THE ANATOMY OF THE CARDIOVASCULAR SYSTEMS OF ANIMALS AND MAN

Wesley D. Anderson*

Aspects of the gross and microscopic anatomy of the cardiovascular systems and associated structures of the dog, sheep, calf and pig were correlated with man. They were: (1) heart, great vessels of the thorax, bronchial arteries, azygos venous systems and arterial supply to the spinal cord; (2) intracranial arterial systems and venous drainage of the head and neck; (3) arterial supply to the ruminant stomach; and (4) arterial patterns to the extremities. The mediastina, pericardium and its sinuses, and valvules and cusps of the heart were similar in sheep, calf and man while the azygos systems, ascending aorta, aortic arch and the course and termination of the coronary arteries differed. The vertebralbasilar system was similar in all four species but the internal carotid system and venous drainage of the head and neck differed markedly in the animals and from man. The arterial supply to the ruminant stomach was from a celiac-cranial mesenteric trunk or from the celiac artery. The rumen received its entire arterial supply from the right and left ruminal arteries and blood supply to the abomasum (true stomach) was from the common hepatic and left gastric arteries. The anatomy of the arterial patterns of limbs of dog and calf was studied with a view to employing them for the study of collateral circulation to the distal limb in the event of proximal ligation or thrombosis. When both femoral arteries were ligated below the origin of the profunda femoris in ten dogs, there was no noticeable effect on limb function nor gross evidence of ischemia or necrosis in the distal limb because of the extensive anastomoses between branches of the profunda femoris, internal iliac and femoral arteries.

INTRODUCTION**

The knowledge of how organs and tissues of animals compare anatomically to those of man is becoming increasingly important today. The selection of experimental animal models is often based in part on the similarity in gross or histological structure between animal and man. Unfortunately the animal body has not been studied anatomically in as great a detail as the human body.

*Department of Anatomy, University of Minnesota Medical School Minneapolis, Minnesota 55455.

** University of Minnesota PH43-68-1377 Vascular Anatomy of the sheep.

The objective of this study is to describe portions of the cardiovascular system in sheep and other quadrupeds and relate these findings to comparable structures in man. The use of the two ruminant species, the sheep and calf in cardiovascular research is becoming increasingly common; therefore, emphasis in this paper will be placed on these two species. Also a knowledge of how the anatomy of these species relates to man is relevant to the eventual goal of cardiovascular research today, namely the application of new knowledge gained from animal experimentation to improve the health and well being of human beings. Directional nomenclature used has been based upon the anatomical position for quadruped mammals which is the erect position in which the animal is standing firmly on thoracic and pelvic limbs with head directed forward. In most cases the terminology of the *Nomina Anatomica Veterinaria* of 1968 used in this study is based upon the anatomical position; however in some cases, as in the case of the coronary arteries, it is not. Plastic cast injections of the cardiovascular system of sheep and other species have documented the course of these and other vessels and thus have been of value in establishing nomenclature for this paper which is comparable to the well accepted NA terminology for human beings. In some cases the older anatomical term has been included in parenthesis and in several cases the comparable human term has likewise been parenthesized.

THORAX

Thoracic Inlet

The bony thorax of many quadrupeds is triangular in shape. In the case of the sheep (Figure 1), it has a narrow spax, the cranial thoracic aperature, and a base more than six times as wide called the caudal thoracic apera-

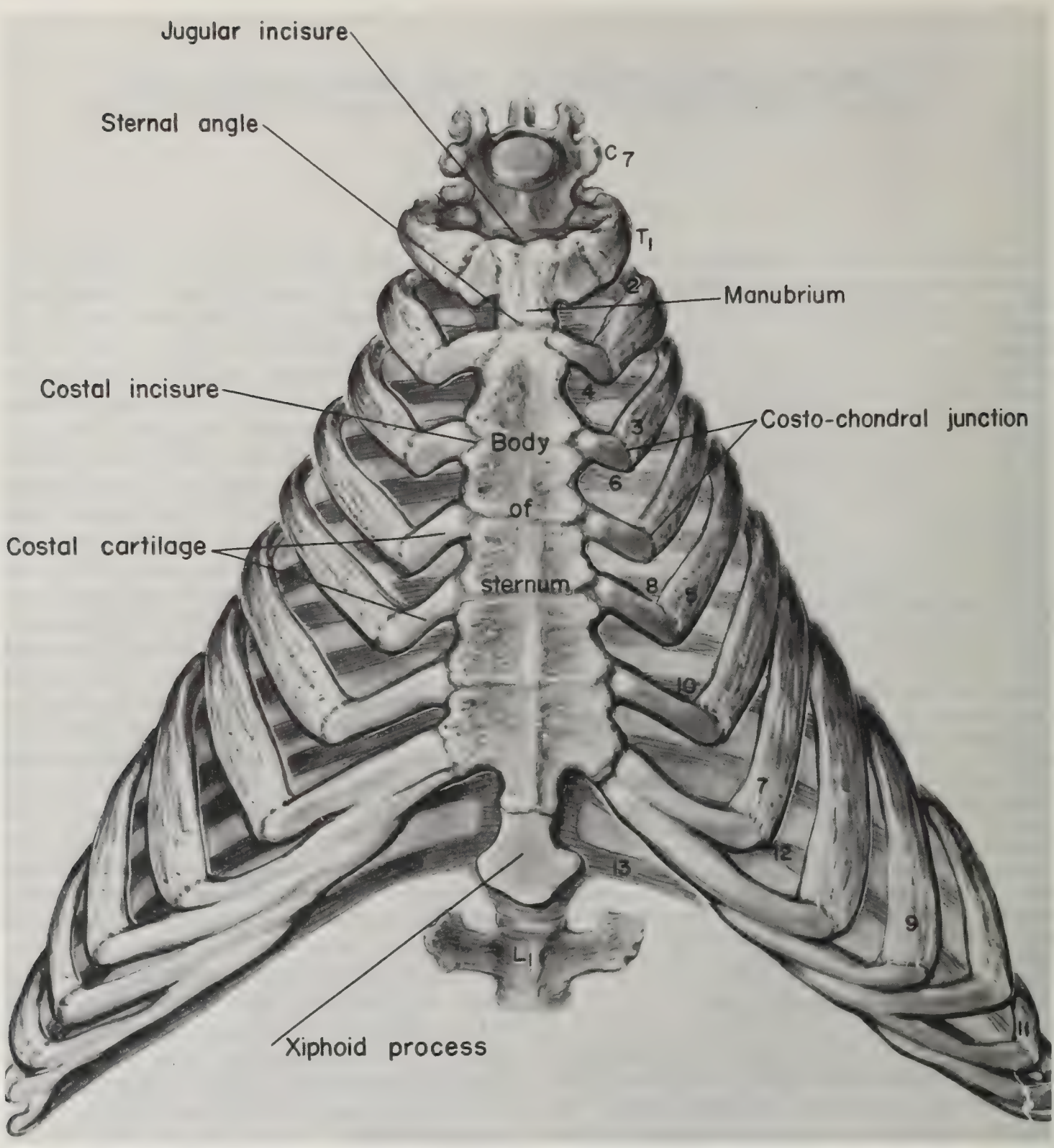


FIGURE 1.—Ventral (anterior) view of skeleton of ovine thorax.

ture. The thoracic inlet in spite of its narrow transverse diameter (5.1 cm mean diameter in adult sheep)[1] and slightly larger ventrodorsal diameter must transmit the same structures that pass in and out of the superior thoracic aperature of man, namely arteries, veins, autonomic and sensory nerve fibers, thymus, lymphatics, esophagus and trachea. A small thoracic inlet in sheep therefore may seriously compromise the use of this animal in the evaluation

of certain artificial heart devices which must be implanted in the cranial portion of the thorax. The sheep does however have many morphological advantages in cardiovascular research; unfortunately, the small thoracic inlet is one of its disadvantages.

The bony thorax of man on the other hand (Figure 2) is more conical in shape and in the adult male the transverse diameter averages 12.5 cm and its anteroposterior diameter measures 6.0 cm.[2]

Trachea, Bronchi and Associated Structures

The bifurcation of the trachea into right and left principal bronchi occurs at the sixth thoracic vertebra in sheep and the seventh thoracic vertebra in the living human being (Figures 3 and 4). The right principal bronchus has a wider diameter than the left bronchus in sheep, calf and man, and the left principal bronchus in these species is larger and arises by more of an acute angle than the right (Figures 5 and 6). There is also similarity in position of the aorta,

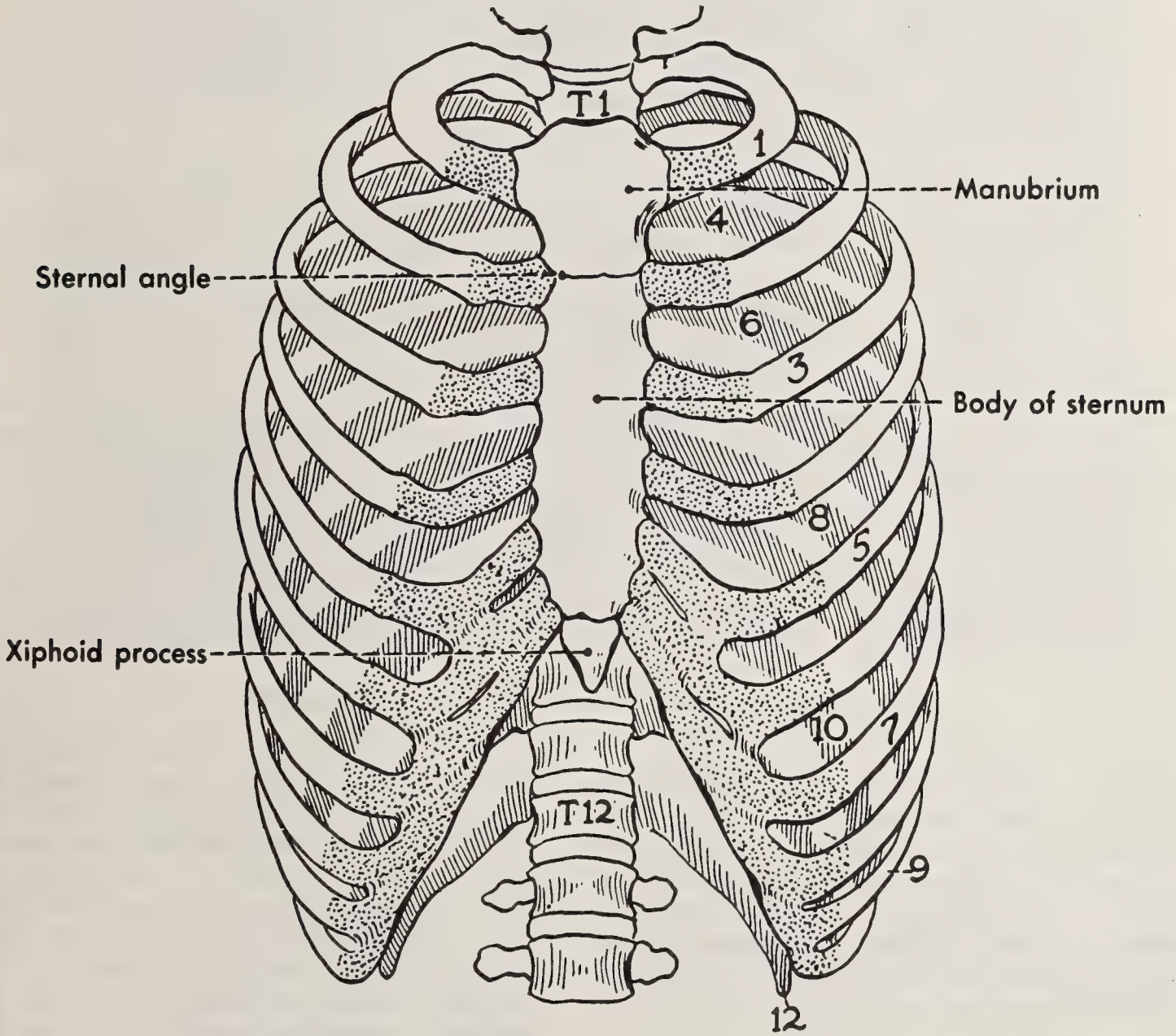


FIGURE 2.—Anterior view of human bony thorax.

Reproduced by permission from: W. Henry Hollinshead. TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

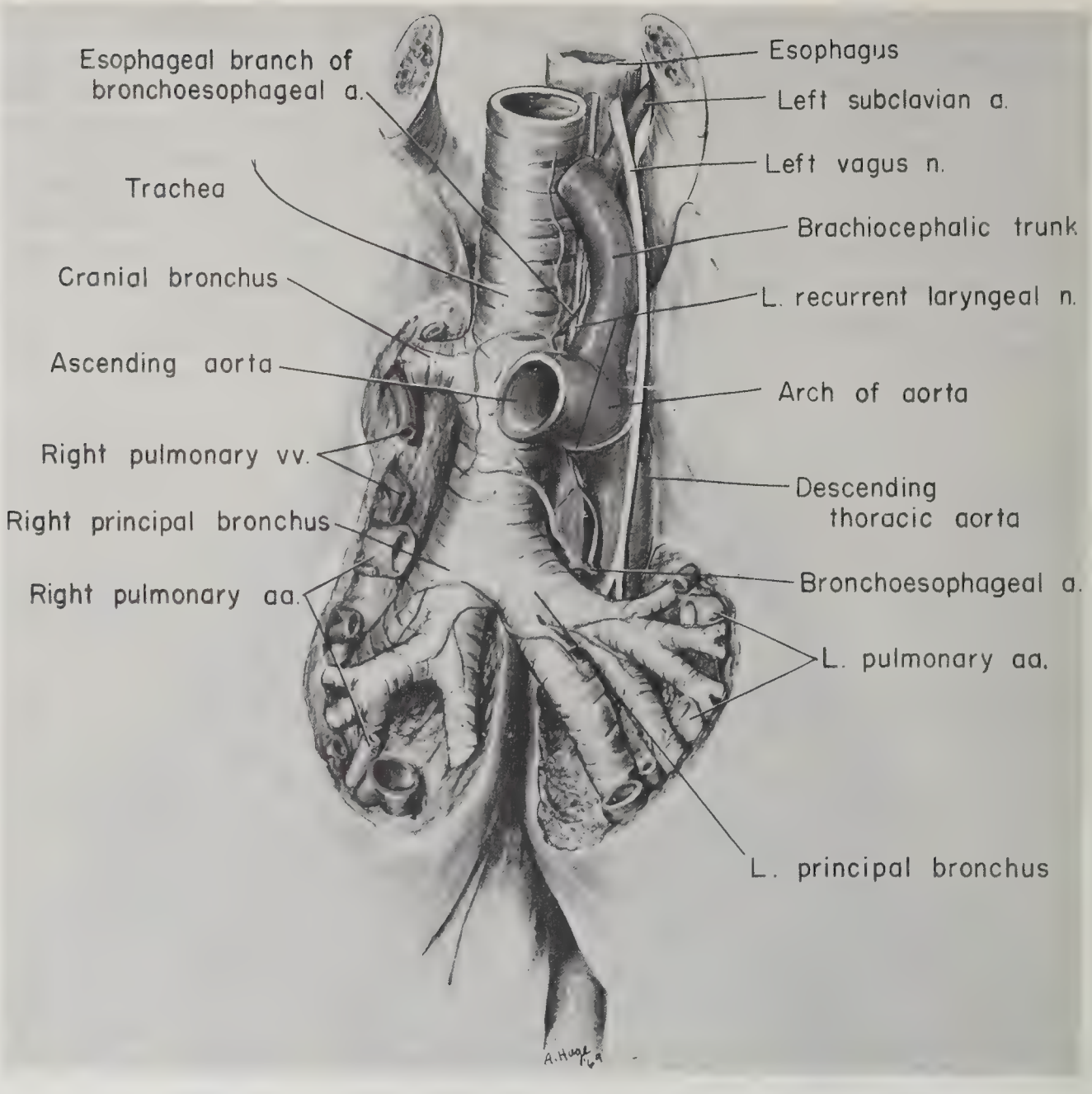


FIGURE 3.—Trachea, bronchi and associated structures of the sheep.

pulmonary artery and veins to the left principal bronchi in sheep and man. The left recurrent laryngeal nerve most commonly recurs around the aortic arch in most mammals and gives origin to caudal or inferior nerves, tracheal and esophageal branches and then ascends in the cervical region on the ventrolateral surface of the esophagus.

Lung

The nomenclature of the lung in quadrupeds and in man is based on division of bronchi rather than external fissures on the lung. All species except the horse and man have four lobes of the right lung. They are: the cranial lobe, middle lobe, caudal lobe, and accessory lobe. In all species, except horse and man, the cranial lobe of the left lung is divided into cranial and caudal parts by an interlobar fissure. In ruminants the right lung is also divided into cranial and caudal parts. The left lung, the smaller of the two lungs of sheep and calf, extends from the thoracic inlet caudally to the 11th intercostal space and the thymus

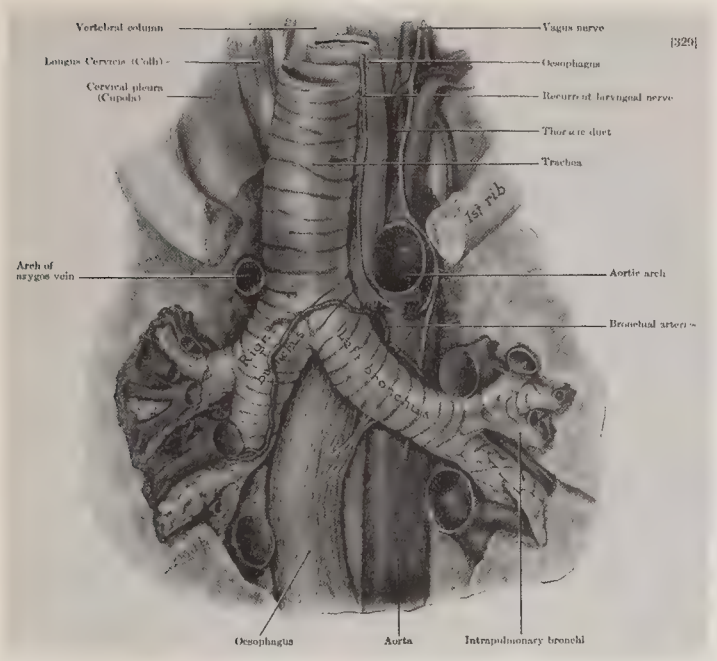


FIGURE 4.—Trachea, bronchi and associated structures of man.

Reproduced by permission from: J. C. B. Grant, AN ATLAS OF ANATOMY (ed. 5). Copyright (1962). The Williams & Wilkins Co., Baltimore.

in the young extends from the neck into the cranial mediastinum. The right lung of sheep and calf is larger than the left lung and is composed of four lobes. They are: the large cranial (formerly the apical lobe) lobe with its cranial portion and caudal portions, the middle lobe (formerly cardiac lobe), the caudal lobe (formerly the diaphragmatic), and the accessory lobe (formerly called the azygos lobe).

Aorta

The ascending aorta of sheep weighing between 120 and 220 lbs., is approximately 2.4 cm in diameter and 1.5–2.5 cm long,[1] while the ascending aorta in man is generally 5–5.5 cm in length[2] (Figures 7 and 8). After the ascending aorta gives origin to the right and left coronary arteries, it ascends cranially and dorsally in the middle mediastinum between the pulmonary trunk and right atrium. The ascending aorta is covered by fibrous pericardium and shares a serous reflection with the pulmonary trunk. It is continued in the cranial mediastinum as the aortic arch and passes upward and slightly to the left. The aortic arch in the sheep and calf is characterized by giving off a single large branch, the brachiocephalic trunk, while in man it most commonly gives origin to the brachiocephalic trunk, left common carotid and left subclavian arteries. Occasionally in sheep, calf and man a small artery (or arteries) originates from the left surface of the aortic arch at the level of the brachiocephalic trunk and descends to ramify over the pericardial sac and atria and is called a "middle coronary" artery.

Variations in the Aortic Arch

Figure 8 shows some variations in the branches of the human aorta. In contrast to the many anatomic variations in the aortic arch in man as previously mentioned, the aortic arch of sheep usually has only a single large branch, the brachiocephalic trunk. The aortic arch of the dog, cat, horse and pig on the other hand most commonly has two branches namely the brachiocephalic trunk and left subclavian artery arising from it.

The Bronchial Arteries

In quadruped mammals the bronchial and esophageal arteries commonly arise from the descending thoracic aorta as a trunk, the bronchoesophageal artery. Occasionally bronchial and esophageal branches will arise individually (Figure 9) which is the case most commonly in man. When bronchial and esophageal branches do not arise from the descending thoracic aorta, they are branches of the dorsal intercostal arteries IV–VI. The bronchial arteries are important nutritive arteries and accompany the bronchi distally as far as the alveolar ducts and supply the bronchioles, the connective tissue septa, vasa vasorum to the walls of the large pulmonary vessels, and the bronchiolar epithelium. In recent years with the advent of heart and lung transplantation and research into the development of cardiac assist devices, the significance of bronchial arteries has been emphasized, and thus they should be preserved in surgical procedures of the thorax. In sheep the bronchial arteries are generally 2–3 mm in diameter and in Figure 9 one is seen arising from the lower surface of the descending thoracic aorta and passing with pulmonary arteries and veins deep into the substance of the

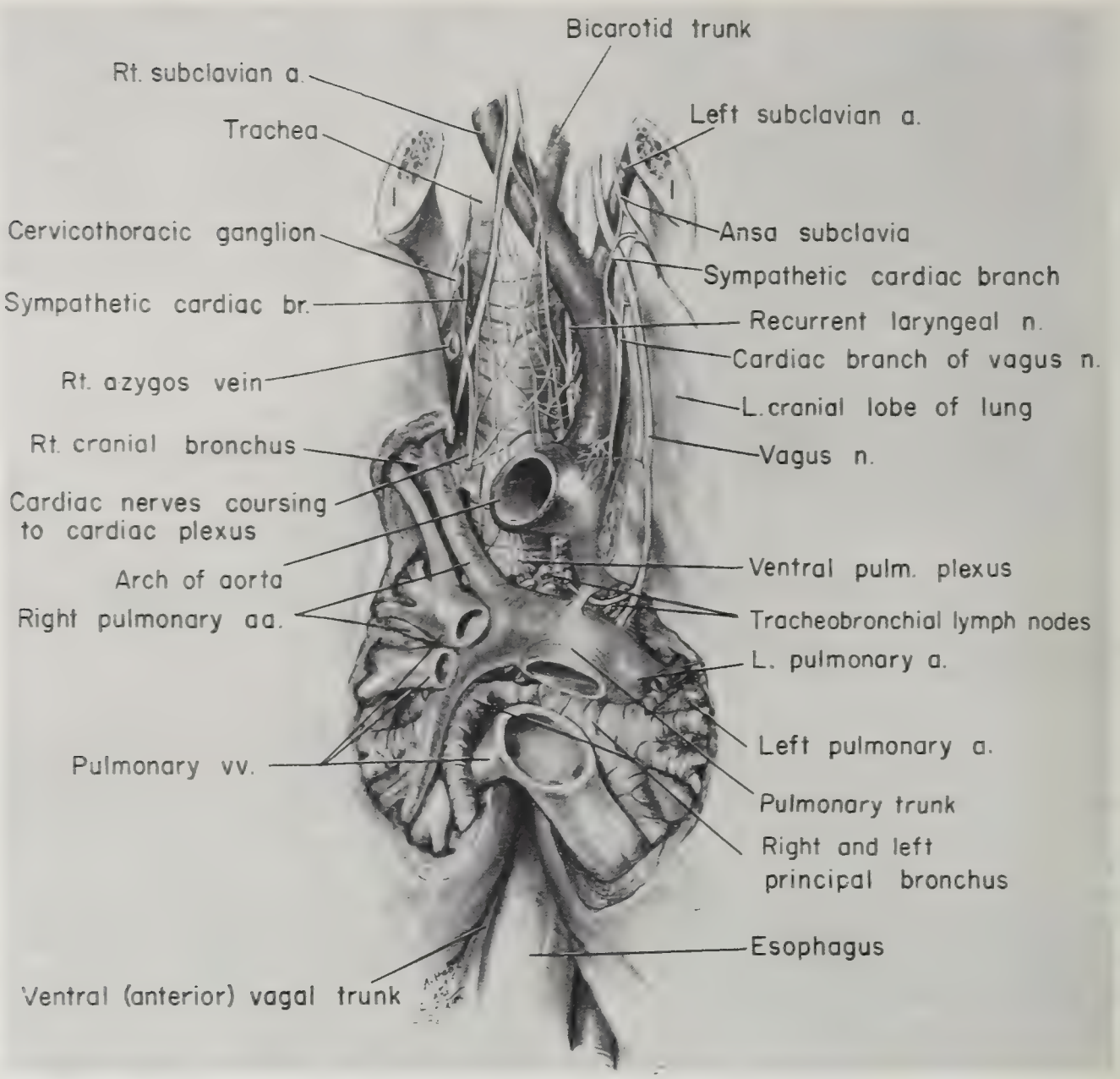


FIGURE 5.—Pulmonary arteries and veins, sheep.

lung. In man there are usually three bronchial arteries, one arising from the right third posterior intercostal artery and two on the left side which arise from the descending thoracic aorta.[3]

HEART

Sternocostal Surface

When the ventral portion of the bony thorax of the sheep is removed and one looks into the pleural cavity, it is evident that the vascular and visceral structures passing through the cranial thoracic aperature compactly fill this narrow space (Figure 10). The superior thoracic aperature of man, as previously noted, is much more spacious and has a greater margin of safety in the event of encroachment upon the thoracic inlet by disease processes or by space occupying prostheses (Figure 11).

The ovine heart, within its pericardial sac, extends from the second intercostal space to the level of the sixth rib. Its apex is directed slightly to the right of the median plane and the pericardial sac is anchored to the sternum by poorly developed fibrous bands, the sterno-

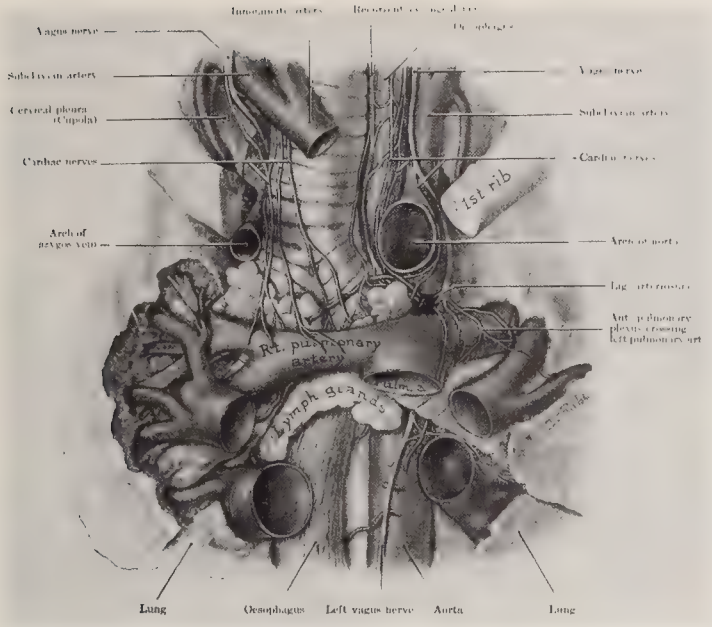


FIGURE 6.—Pulmonary arteries and veins, man.

Reproduced by permission from: J. C. B. Grant, AN ATLAS OF ANATOMY (ed. 5). Copyright (1962). The Williams & Wilkins Co., Baltimore.

pericardial ligament. The sternocostal surface of the sheep heart is partially covered by the right and left cranial lobes and right middle lobe of the lung (Figure 10), and in the young, the heart is also partially covered by the thoracic lobe of the thymus. The fibrous pericardium is coated by a layer of mediastinal pleura and is crossed near the base of the heart by the right and left phrenic nerves. Cranial to the heart in the sheep and calf, the mediastinum encloses considerable amounts of adipose tissue as well as the thoracic lobe of the thymus.

External Anatomy

The surface of the sheep heart, unlike the surface of the human heart, is characterized by abundant amounts of epicardial fat especially prominent in all sulci. The right or acute margin is demarcated by the right marginal artery while the left or obtuse margin does not

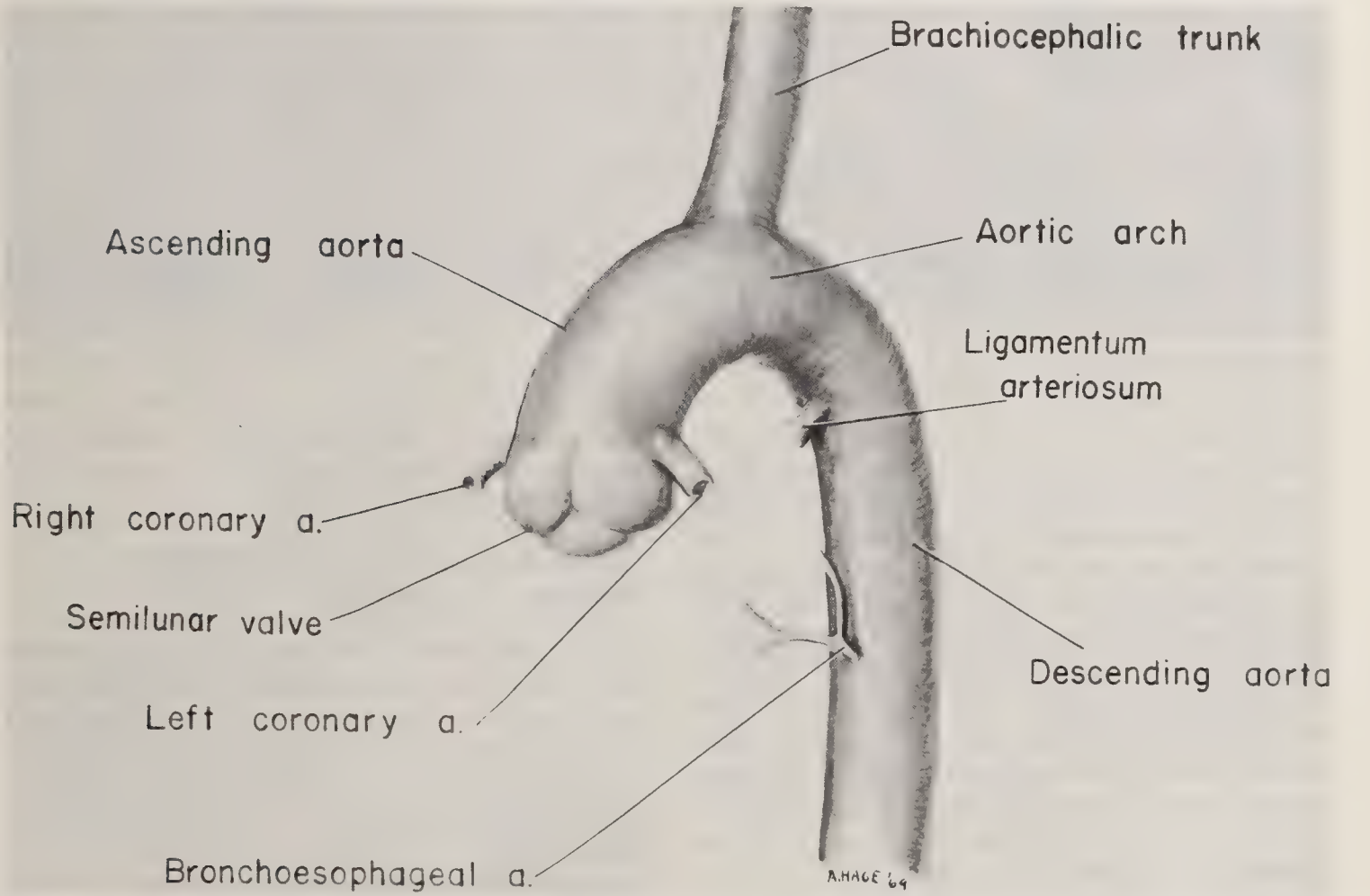


FIGURE 7.—Aortic arch of the sheep.

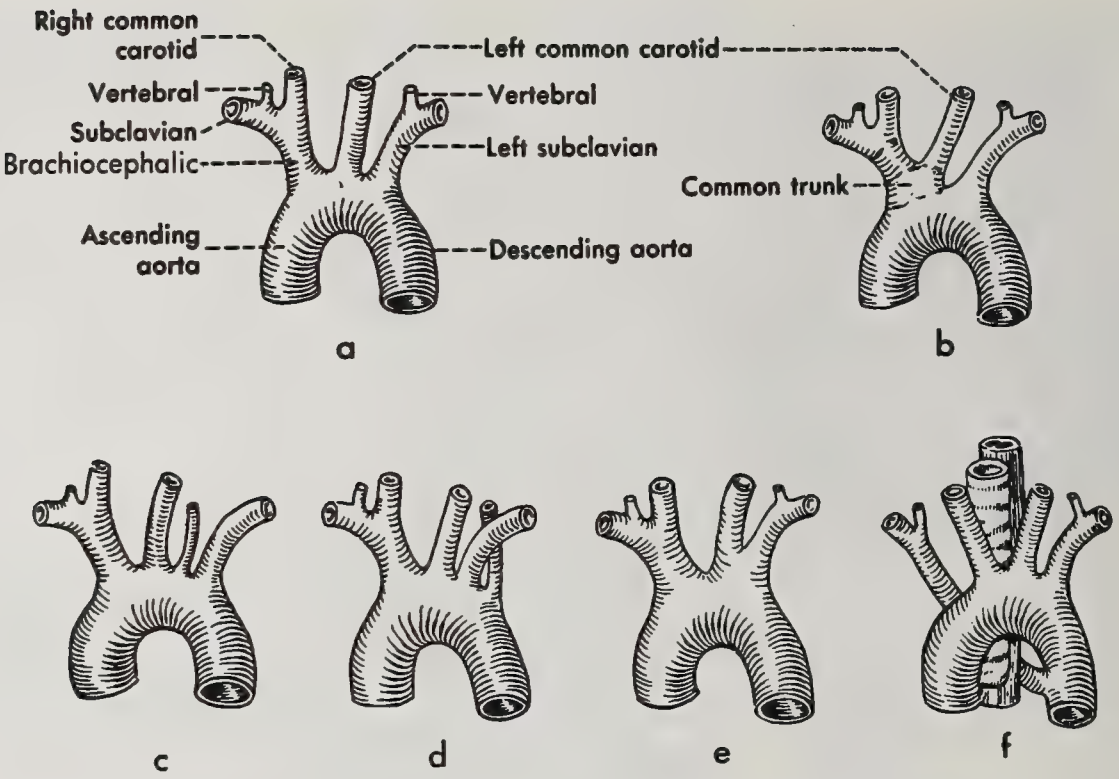


FIGURE 8.—Some variations in the branches of human aorta. *a* is the expected pattern; in *b* the left common carotid shares a short common trunk with the brachiocephalic; in *c* and in *d* the left vertebral artery arises directly from the aorta; in *e* there is a short left brachiocephalic trunk; and in *f* the right subclavian arises anomalously and passes behind trachea and esophagus.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

have a distinct vessel demarcating it. The apex of the sheep heart is somewhat pointed and projects obliquely downward and toward the diaphragm near the sixth intercostal space, while the apex of the human heart projects about eight cm to the left at the fifth intercostal space. The atria and origin of the great vessels form the base of the heart in the sheep while the sternocostal surface is formed principally by the left ventricle and by a portion of the right ventricle. In this sulcus dividing the two ventricles, the cranial interventricular branch of the left coronary artery descends while the great cardiac vein ascends and turns to the left to enter the coronary sulcus where it will become the coronary sinus and empty into the right atrium.

Comparison to Man

The mean distance from the base to apex of the sheep heart is generally 12–13 cm, while the circumference taken at the coronary groove is approximately 29 cm. The distance from base to apex of the sheep heart is remarkably similar to base-apical distance in man of 12.5 cm.[2] The mean weight in a study involving 54 sheep hearts was 222.0 gms, while the average weight of the human female heart is 255 gms and the human male heart 312 gms.[1]

Autonomic Innervation of Heart and Associated Structures

In quadrupeds as in man two sets of nerves reach the heart: one from the sympathetic trunk and one from the vagi. The right and left vagal nerves enter the thorax of the sheep on the lateral surface of the esophagus, cross beneath the subclavian artery and continue caudally parallel to the brachiocephalic trunk where they each give off cardiac branches (Figures 12 and 13). On the left surface of the aorta, the left recurrent laryngeal nerve arises



FIGURE 9.—The bronchial artery documented by plastic injection of the arterial system.

from the vagus, passes around the arch and ascends on the medial surface of the brachiocephalic trunk beneath the subclavian artery to enter the carotid sheath in the caudal cervical region. In man, however, both recurrent laryngeal nerves lie outside of the carotid sheath and generally ascend in the neck in the tracheosophageal groove. In the ruminant the vagus, after giving off the recurrent laryngeal nerve, continues along the ventrolateral surface of the descending thoracic aorta and at the hilus of the lung gives off small branches to the pulmonary, aortic, esophageal and cardiac plexuses, and some fibers directly to the heart. Near the hilus of the lung each vagus bifurcates into a ventral and dorsal trunk.

Sympathetic Nerves

The thoracolumbar division of the autonomic nervous system is represented in the thorax by a chain of sympathetic ganglia embedded in endothoracic fascia and located lateral to the vertebral bodies and ventral to the angle of the ribs (Figures 12 and 13). In sheep, as in man, the first thoracic sympathetic ganglion and caudal cervical sympathetic ganglion are commonly fused into a large ganglion called cervicothoracic, or because of its star-shape, the stellate

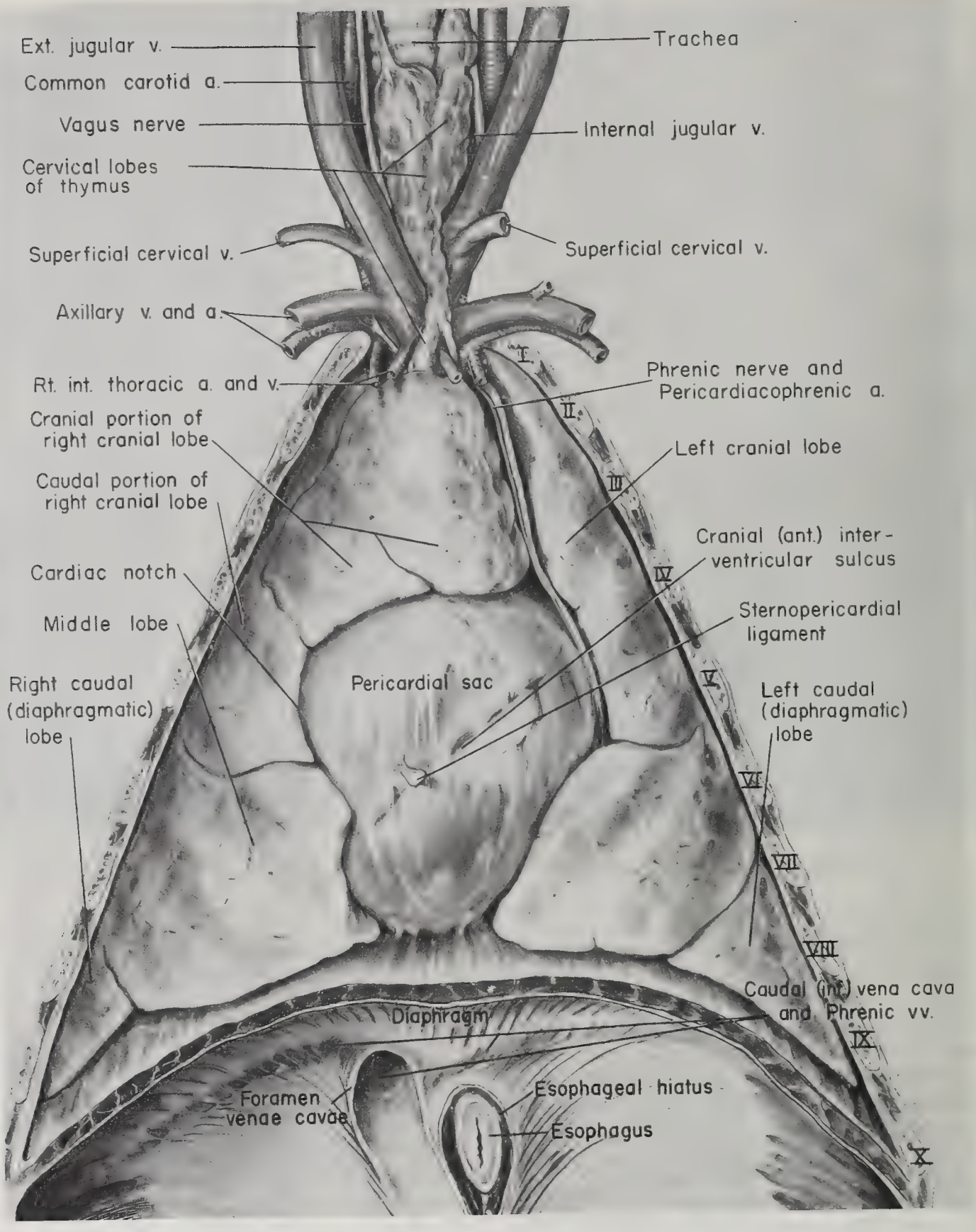


FIGURE 10.—Sternocostal surface of the sheep heart and lobes of the lungs.

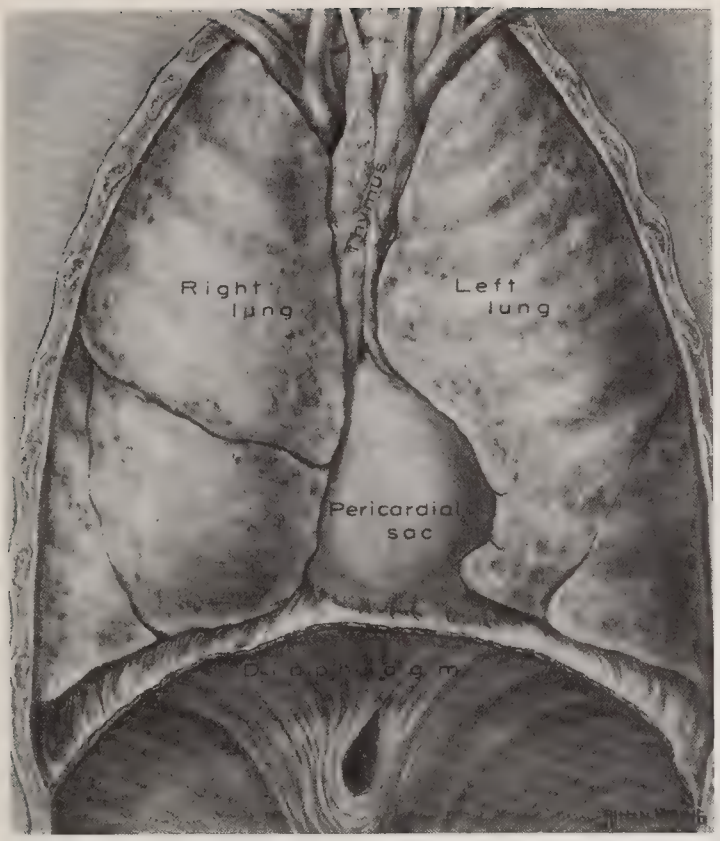


FIGURE 11.—Sternocostal surface of human thoracic viscera in situ.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

ganglion. The vertebral nerve arises from the cervicothoracic ganglion and dips beneath the brachial plexus to enter the transverse foramen with the vertebral artery. Other small nerves pass directly from the cervicothoracic ganglion to the lateral surface of the trachea and course beneath the costocervical trunk to unite as a small ganglion on the dorsolateral surface of the trachea, termed the middle cervical ganglion (Figures 12 and 13). In the sheep and some other ruminants, the sympathetic trunk joins the vagus in the cervical region and is continued as the vagosympathetic trunk.

Coronary Arteries and Cardiac Veins

In man and other mammals the right and left coronary arteries are named for their origins from the right and left aortic sinuses and their course and termination on their respective surfaces of the heart.

The left coronary artery, which supplies the majority of the heart in ruminants and dog, emerges from behind the left side of the pulmonary trunk and bifurcates into a descending branch, the cranial interventricular artery and a circumflex branch. After arising from the ascending aorta at the left aortic sinus, the left coronary artery in the dog often gives origin to a septal branch to the interventricular septum. The septal artery when it arises at this position presents a technical problem when one is desirous of placing an electromagnetic blood flow probe around the trunk of the left coronary for direct and continuous blood flow measurements (Figure 14). The cranial interventricular artery in quadrupeds descends in its sulcus with the great cardiac vein to the apex of the heart where it often enters into anastomoses with the caudal interventricular artery descending from the caudal (posterior) surface of the heart. The circumflex branch courses to the left in the coronary sulcus with the great cardiac vein and gives origin to the left marginal and caudal ventricular arteries. In ruminants and the dog, the left coronary artery terminates on the caudal surface of the heart as the caudal interventricular artery (Figure 15) while the left coronary artery in man most commonly ends on the posterior (diaphragmatic) surface of the heart as the left ventricular artery. Thus the majority of the front as well as the back surface of the ruminant and dog heart is supplied by the left coronary artery (Figure 15), while the majority of the posterior surface of the human heart is usually supplied by the right coronary artery. The left coronary artery of the dog is larger and supplies a greater amount of total myocardium than the right coronary artery. In dogs weighing 13–18 kg an electromagnetic blood flow transducer with a luminal diameter of 2.5–3 mm will usually be satisfactory in obtaining blood flow measurements from the left coronary artery. The cranial interventricular branch of the left coronary in the dog is approximately the same diameter as the circumflex branch and is approximately seven cm long. It has many tiny ventricular branches in addition to a larger left ventricular artery at the apex of the heart and is similar in this respect to man (Figure 16).

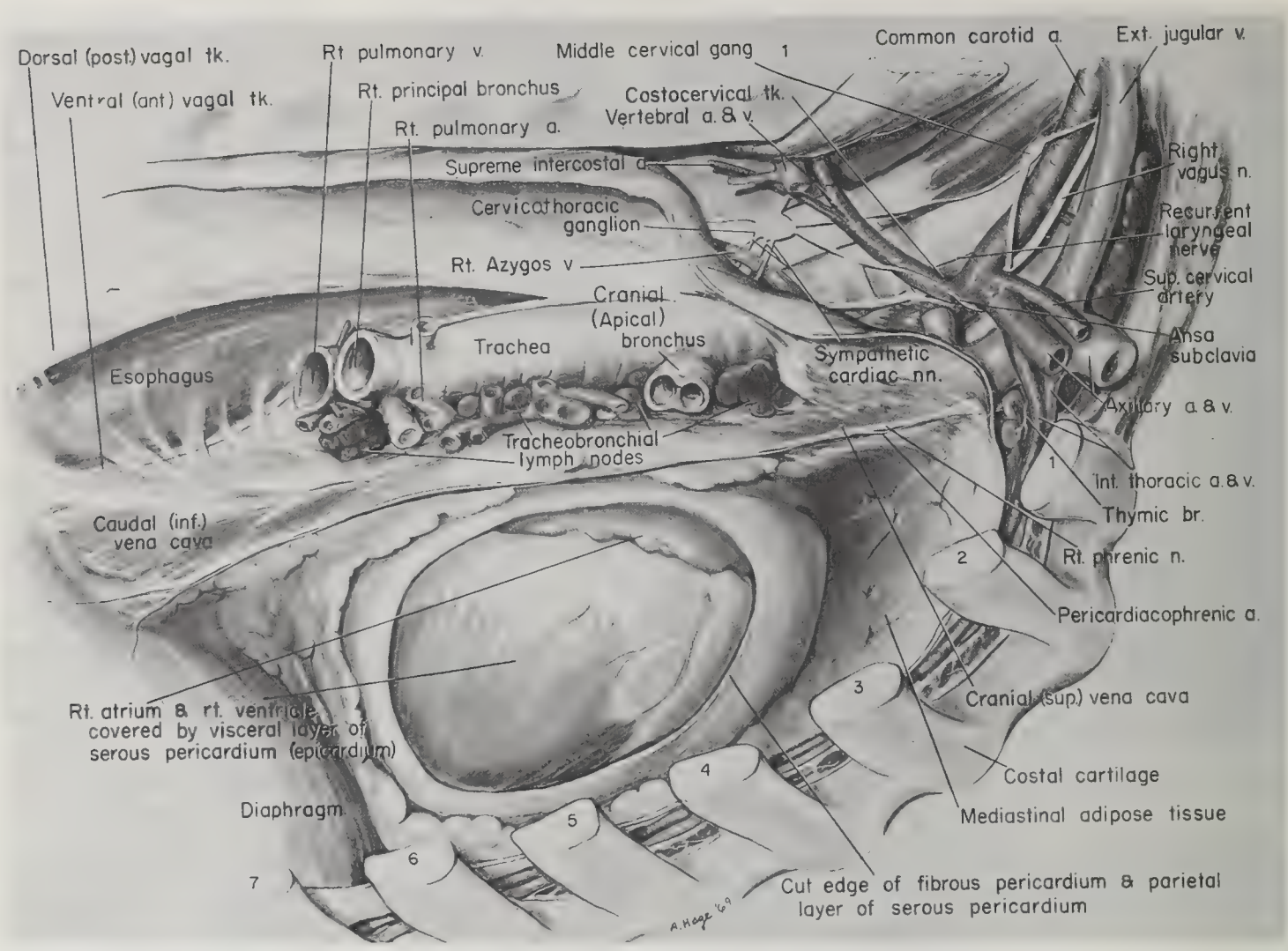


FIGURE 12.—Right surface of heart, great vessels and associated thoracic structures, sheep.

The right coronary artery in mammals arises from the ascending aorta within the right aortic sinus, emerges deep to the right side of the pulmonary trunk and courses around to the back of the heart in the coronary sulcus. It gives origin to tiny descending myocardial branches, a larger right marginal artery on the acute surface of the heart, and in sheep, calf and dog terminates on the caudal surface as small unnamed branches which descend toward the apex of the heart. In man, cat and horse however the right coronary artery terminates on the posterior surface of the heart as the posterior interventricular artery (Figure 17).

The majority of the cardiac veins parallel coronary arteries in most quadrupeds like they do in man (Figure 18). The largest tributary of the coronary sinus in most mammals, the great cardiac vein, ascends from the apex of the heart in the interventricular sulcus and near the origin of the circumflex branch of the left coronary artery it turns sharply to the left and passes around the heart in the coronary sulcus. Also in most animals, as is the case in man, the tiny oblique vein of the left atrium marks the change in name from the great cardiac vein to coronary sinus (Figure 19). Of considerable importance to the investigator sampling venous blood from the myocardium is that in the sheep and calf the coronary sinus is joined by the left azygos (formerly called the hemiazygos) vein before emptying into the right atrium. Therefore one sampling venous blood from the terminal portion of the coronary sinus in these species is sampling venous return from the thoracic wall and abdomen as well as from the myocardium.

The internal anatomy of the heart of sheep,

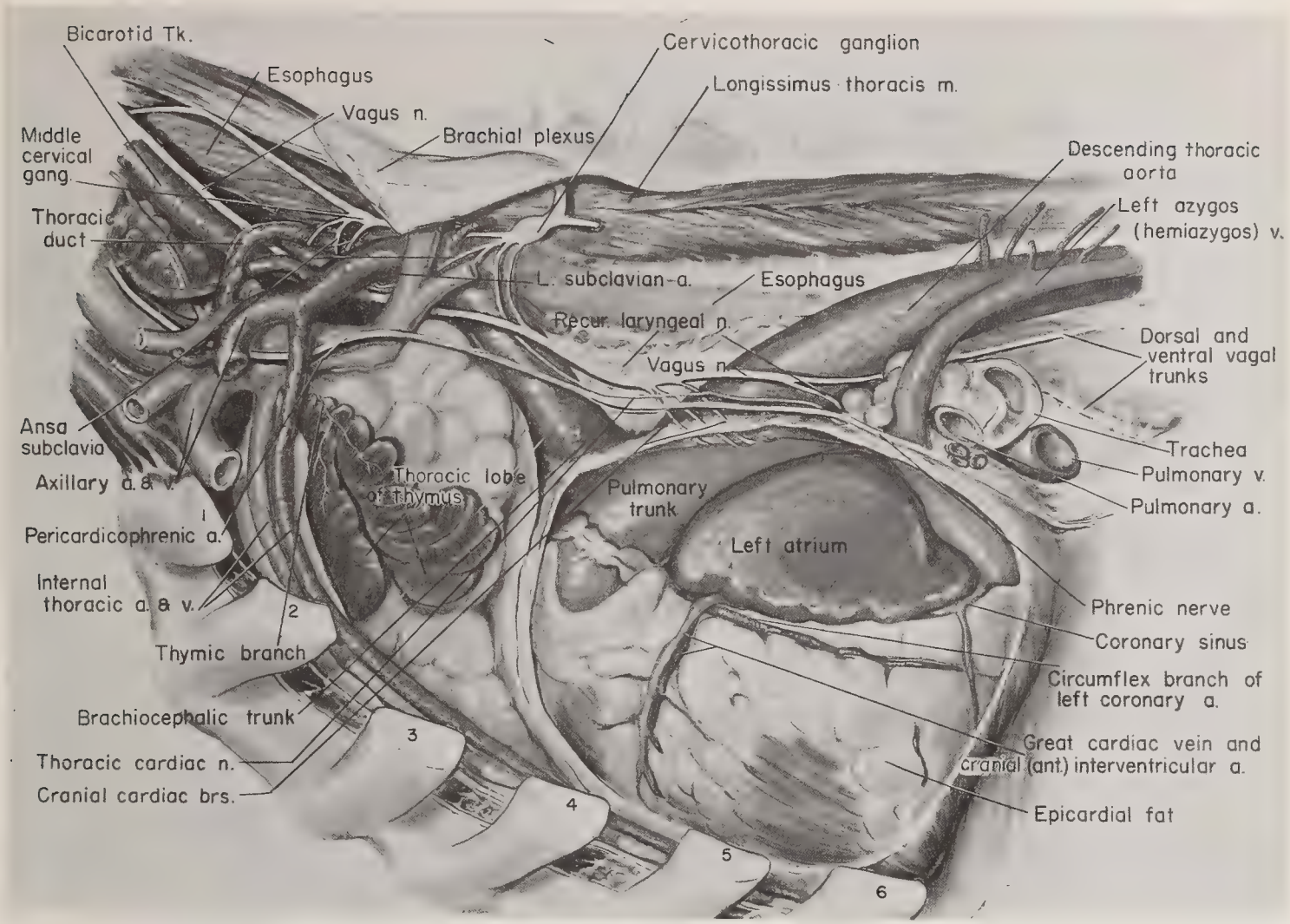


FIGURE 13.—Left surface of heart, great vessels and associated structures, sheep.

calf, dog and other quadrupeds is similar to the well understood internal structure of the human heart. All normal (and abnormal) ostia found in the heart of man can usually be found in the animal heart. Of special significance is the marked similarity between valvules and cusps of sheep and human hearts (Figures 20 and 21).

Pericardial Sinuses

The two sinuses of the pericardial cavity are the transverse and oblique. The latter sinus is merely a blind pouch and has no clinical significance while the transverse pericardial sinus does as it may be used surgically as a channel to exert digital or sutural compression upon the pulmonary trunk and ascending aorta to temporarily halt blood flow into the systemic and pulmonary circuits. When the pericardial sac is opened one notes that the visceral layer of pericardium (epicardium) is reflected around both pulmonary trunk and ascending aorta in a manner to completely encircle both vessels. This space remaining in the pericardial sac behind the two great vessels is the transverse pericardial sinus. Figures 22 and 23 illustrate the comparative features of the pericardial sinuses in sheep and man.

The Azygos and Hemiazygos Venous Systems

In ruminants and in the pig, small ascending lumbar veins course high in the sublumbar region and unite with small subcostal veins to form the left azygos vein (also called the hemiazygos vein. The left azygos vein continues cranially invested in endothoracic fascia deep to the parietal pleura, and at each intercostal space is joined by dorsal intercostal veins.

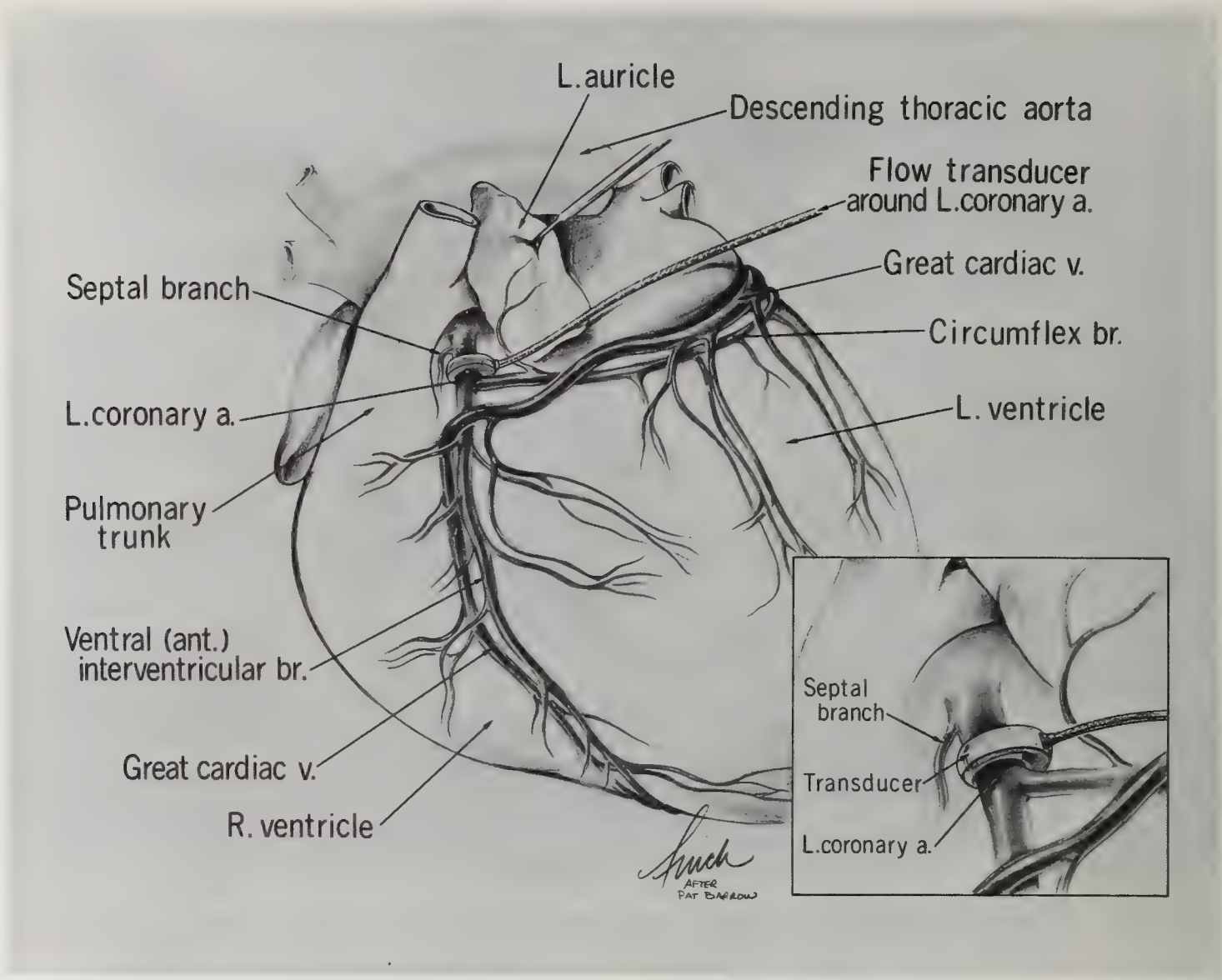


FIGURE 14.—Left coronary artery (dog) showing placement of an electromagnetic blood flow probe (BioTronex) around the origin of the artery.

When the left azygos vein reaches the level of the fifth to sixth thoracic vertebra (a position cranial to the hilus of the lung), the vein turns ventrally and empties into the coronary sinus (Figure 24). As the left azygos ascends on the dorsal thoracic wall, it is located on the left side of the descending thoracic aorta.

The right azygos vein (formerly the azygos vein) is poorly developed in sheep, calf and pig and arises by small dorsal intercostal tributaries at the fifth and sixth thoracic vertebrae and continues cranially receiving dorsal intercostal veins at each intercostal space. After receiving its last dorsal intercostal vein it turns sharply downward and empties into the cranial vena cava at the level of the second thoracic vertebra (Figure 25).

In man the hemiazygos, accessory hemiazygos and right superior intercostal veins empty into the azygos vein, while the left superior intercostal, although communicating with the accessory hemiazygos vein, commonly drains into the left brachiocephalic vein (Figure 26).

The Clinical Significance of Azygos Systems

The azygos systems drain the thoracic wall and, by their connections to the lumbar veins, assist in the drainage of the abdominal wall. In the event of thrombosis or ligation of the caudal (inferior) vena cava below the level of the

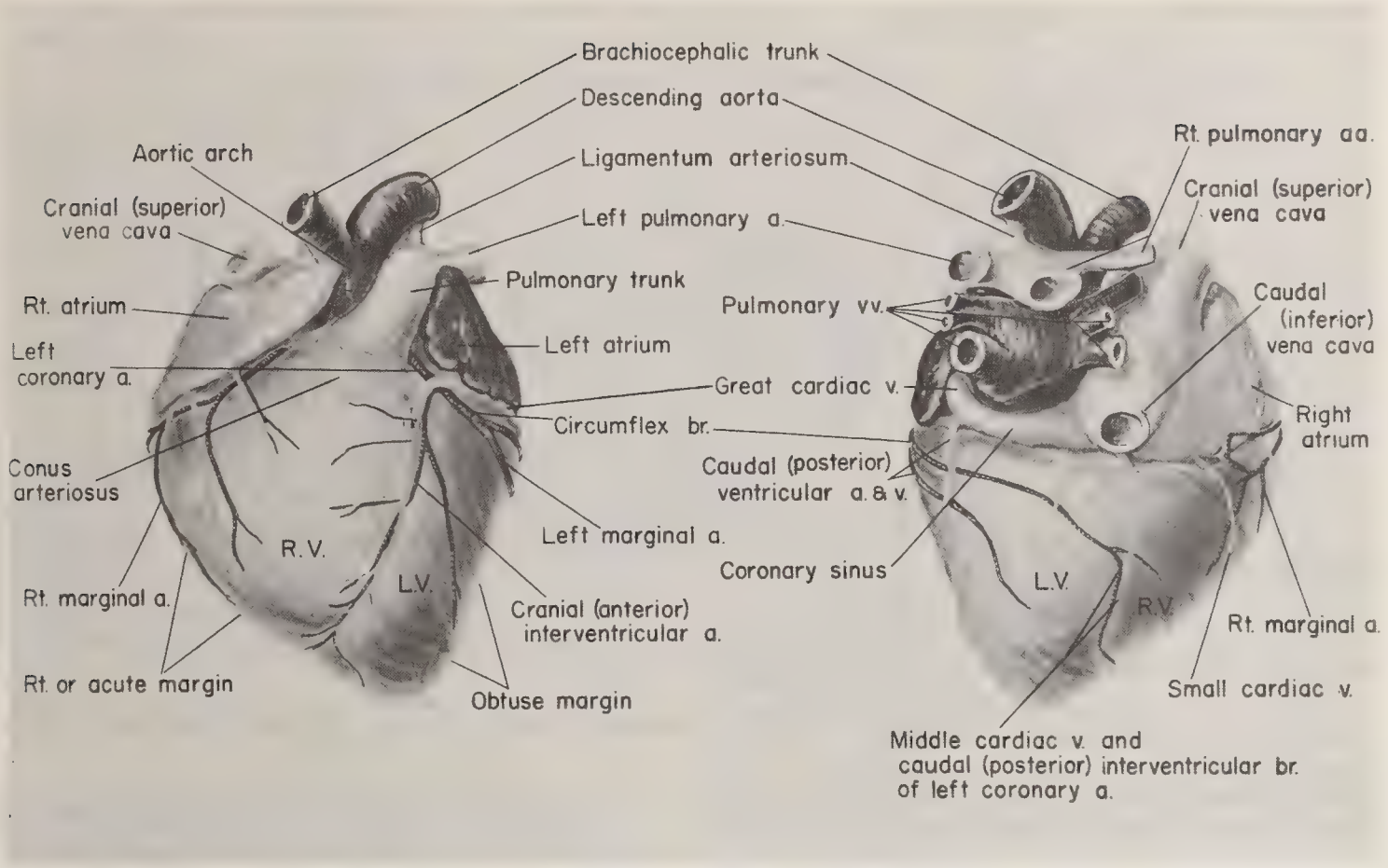


FIGURE 15.—Sternocostal surface of ovine heart showing coronary arteries and great cardiac vein (left) and caudal (posterior) surface of ovine heart demonstrating terminations of coronary arteries and coronary sinus (right).

renal arteries, the azygos systems become important collateral routes for return of venous blood from the caudal or lower extremities to the right atrium.

ARTERIAL SUPPLY TO THE SPINAL CORD

In the thoracic region, aortic aneurysm or aortic bypass may seriously compromise blood supply to the spinal cord. Infarction of the spinal cord in human beings with cardiovascular disease has been reviewed by Henson and Parsons,[4] and in recent years innovations in diagnostic neuroradiology and microsurgery have initiated a resurgence of interest in spinal cord circulatory dynamics which have resulted in a greater appreciation of vascular pathology. As a result of the renewed awareness of the anatomy of the arterial supply to the spinal cord, Burton[5] has expressed the opinion that the incidence of spinal cord vascular disease in man remains to be determined.

In quadrupeds, the ventral spinal artery arises at the level of the atlanto-occipital articulation and courses caudally in the median ventral fissure of the spinal cord. The two dorsal spinal arteries, which also arise from the vertebral arteries, are smaller than the ventral spinal and contribute less blood flow to the cord. The ventral spinal (as is the case for the anterior spinal artery in man) is dependent upon reinforcing radicular branches from the vertebral and dorsal intercostal arteries for adequate perfusion of the spinal cord. Figure 27 is an arterial cast of the arterial supply to the cervical segments of the spinal cord of the sheep. The vertebral arteries give off spinal branches which at segmental levels terminate in radicular arteries in most mammals. The radicular branches anastomose with the small ventral spinal artery and thereby reinforce the arterial perfusion to the cervical segments of the spinal cord. Unfortunately the spinal and radicular

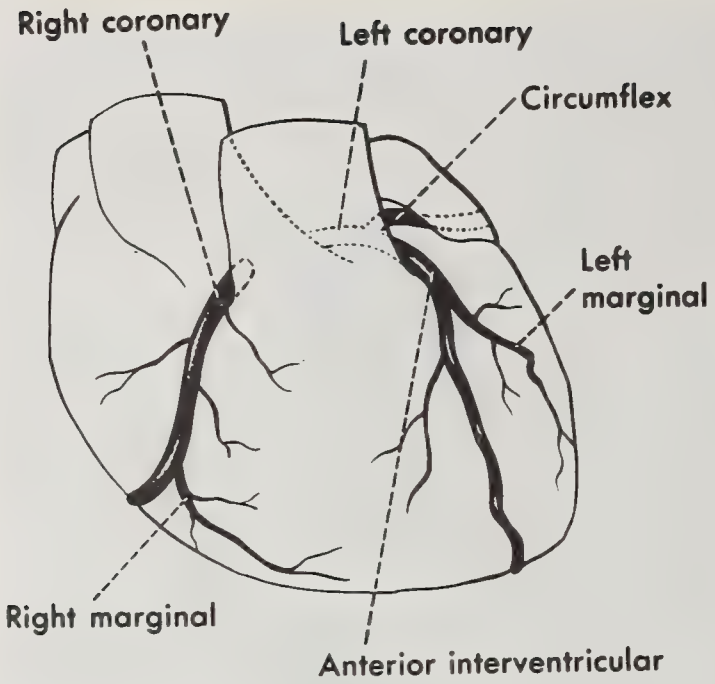


FIGURE 16.—The coronary arteries on the sternocostal surface of the human heart. The right and left marginal arteries are unnamed in the N.A.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

branches are not equally well developed at all segments and this situation results in some portions of the cervical spinal cord being adequately perfused and other segments somewhat poorly perfused.

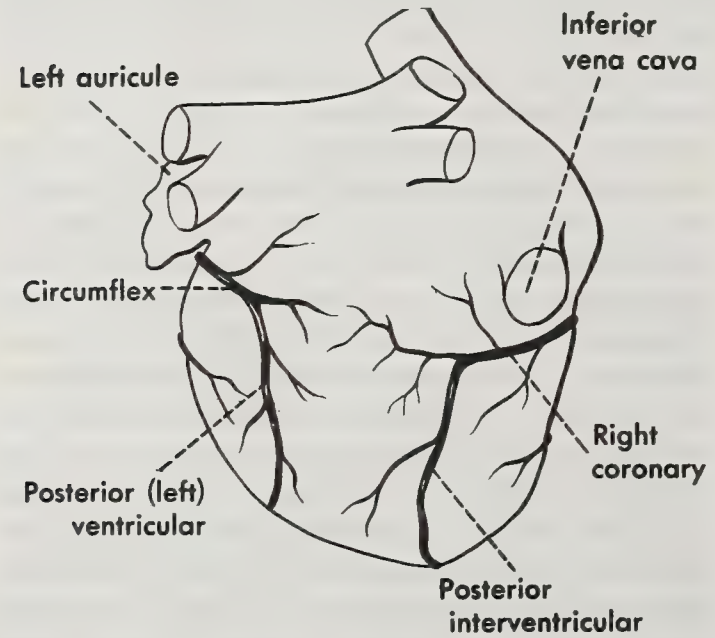


FIGURE 17.—Termination of the right coronary artery on the posterior surface of the heart (man).

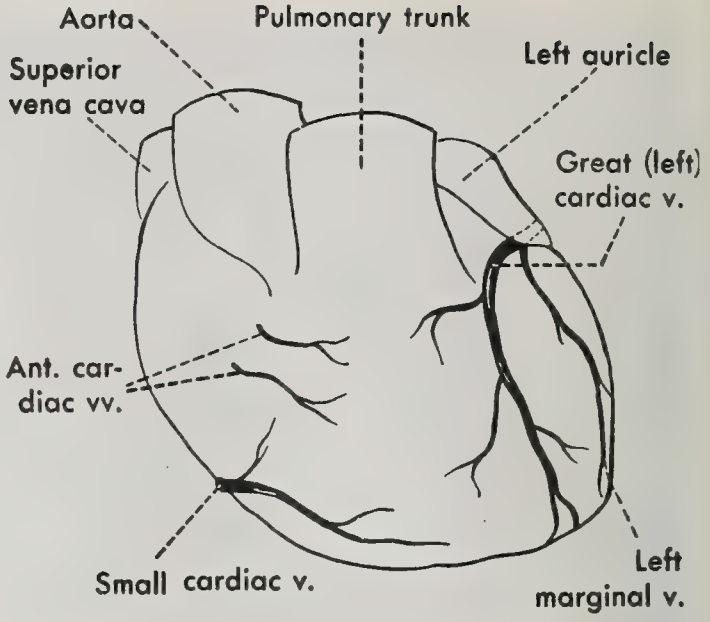


FIGURE 18.—The cardiac veins in an anterior view of the heart. The left marginal vein is not named in the N.A.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

In the thoracic region of the spinal cord, the first two intercostal spaces are supplied by intervertebral branches of the costocervical artery, the deep cervical artery and the dorsal intercostal artery. The rest of the intercostal

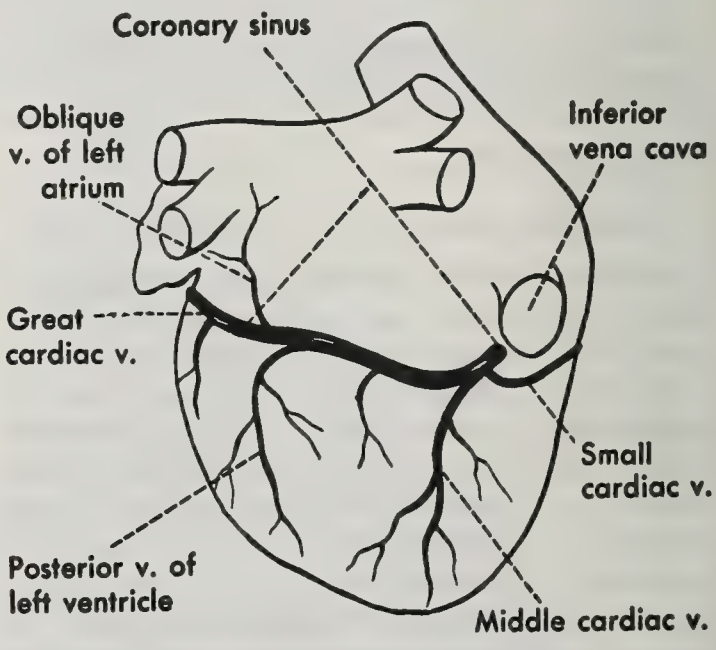


Figure 19.—The cardiac veins and coronary sinus in a posterior view of the human heart.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

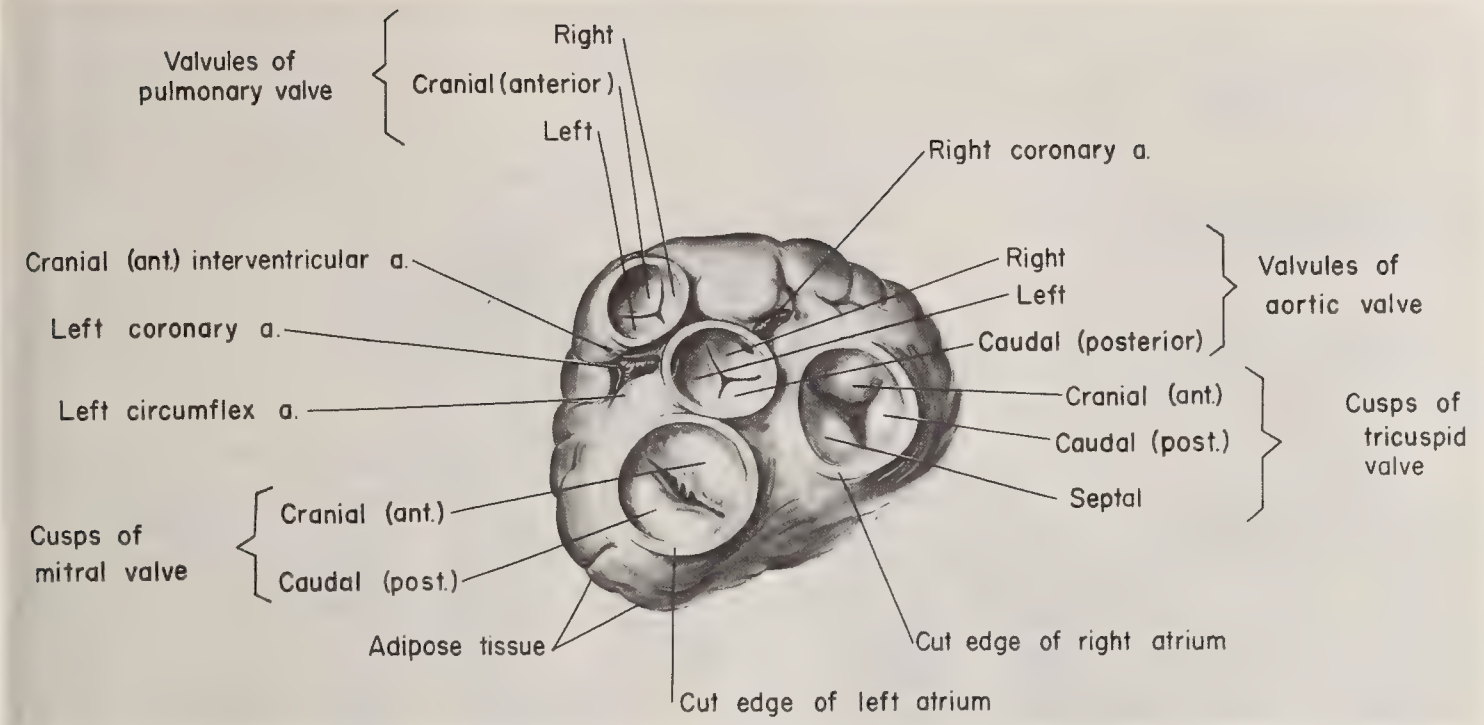


FIGURE 20.—Dorsal (superior) view of semilunar and atrioventricular valves of sheep heart.

spaces are supplied by spinal branches from the aortic intercostal arteries (Figure 28) which anastomose with intercostal branches of the internal thoracic arteries. Each aortic (dorsal) intercostal artery also has a spinal branch which enters the intervertebral foramen with the spinal nerve where it gives off a ventral (anterior) and a dorsal (posterior) radicular artery which penetrates the dura meter. While there is a radicular artery for each spinal nerve root, many of the radicular arteries are small and exhaust themselves in the nerve roots. The radicular arteries, of which there are usually six to eight pairs larger than 300 microns, are important sources of collateral circulation to the ventral spinal arteries. They are: one pair in the upper cervical area; one to two pairs in the lower cervical area; one to two pairs in the upper thoracic area; none to one pair in the middle thoracic area; and one pair in the lower thoracic region.

In the lumbar region the intervertebral branches of the lumbar and sacral arteries furnish radicular branches to the anterior spinal artery.

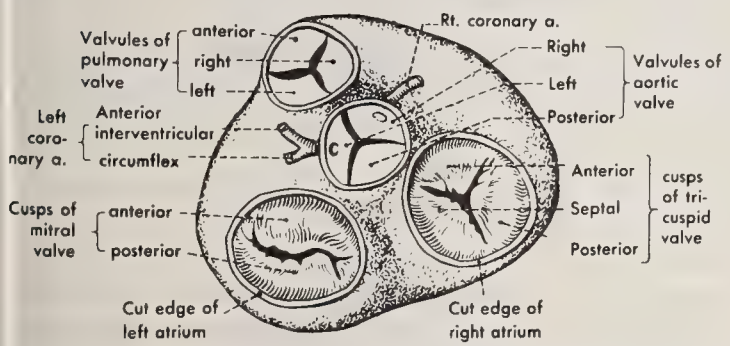


FIGURE 21.—Diagram of the valves of the heart, in a view from above, with both atria cut away and the aorta and the pulmonary trunk cut a little above the levels of their valves.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

THE ANATOMY OF THE VERTEBRAL-BASILAR SYSTEM OF DOG IN RELATION TO MAN AND OTHER MAMMALS

According to Rittman and Smith [6] more than two-thirds of the arterial blood reaches the dog brain by way of the vertebral-basilar system, yet the relatively few accounts of the anatomy of this system in the dog mention only its most striking features.[7–9]

The major arterial system to the brain in 30 dogs was studied following perfusion of the arterial system with neoprene latex and with

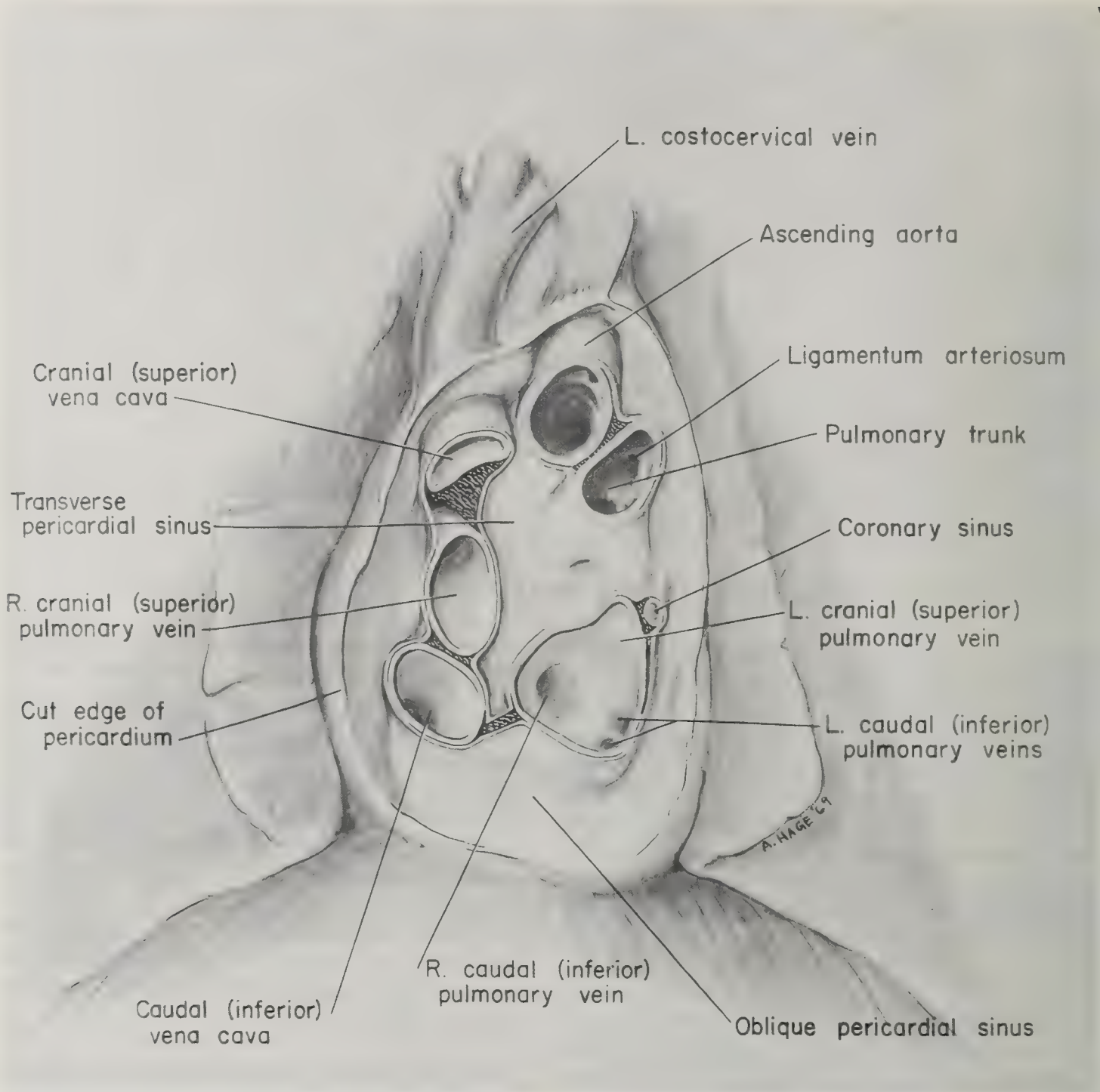


FIGURE 22.—Attachments of base of heart to dorsal (posterior) wall of pericardial sac and pericardial sinuses.

methyl methacrylate* polymer in an additional five dogs. Nomenclature for the intracranial arteries of the dog used in this study corresponds in most cases to the 1968 *Nomina Anatomica* terminology (Table I).

Formation of the origin of the basilar artery was from the right and left vertebral arteries and ventral spinal artery (Figure 29). The vertebral arteries, after arising from the subclavian, ascended in the transverse foramina of the cervical vertebrae like their homologues in man. Near the intervertebral foramen of the atlas, each vertebral artery anastomosed with a branch of the occipital and then passed

* Manufactured by the Imperial Chemical Industries, Ltd. (Great Britain).

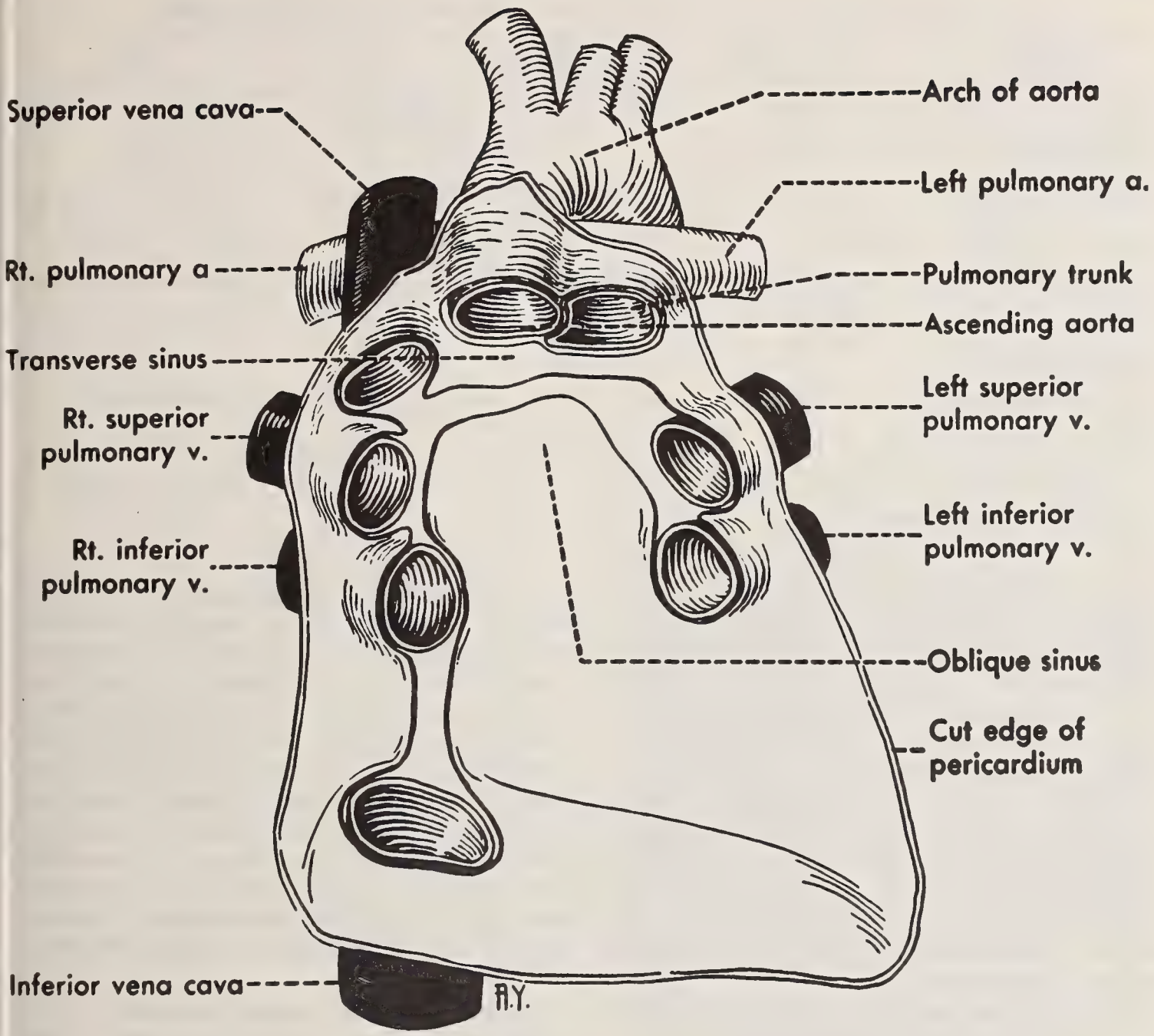


FIGURE 23.—Human pericardial sinuses and pericardial attachments.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

through the intervertebral foramen into the vertebral canal. The vertebral arteries and the relatively large ventral spinal artery formed a spinal arterial circle at the level of the atlanto-occipital articulation. The rostral portion of the circle generally extended a short distance into the cranial cavity and continued as the basilar artery. The branches of the intracranial portion of the vertebral-basilar system of the dog and the portions of the brain and spinal cord supplied by this system are listed in Table II.

The dorsal spinal arteries usually arose from the vertebral arteries and descended on the upper surfaces of the spinal cord.

The posterior inferior cerebellar arteries arose either from the vertebral arteries (as they do in man [10]) or from the basilar. In this study they were traced to the lower and caudal lobules of the cerebellum listed in Table II.

TABLE I.—*Nomenclatures for the vertebral-basilar systems in the dog, cat, fox, and man*[11]

Dog This paper ('71)	Dog Miller, Christensen, and Evans ('64)	Cat Bernstein and Silverstein ('66)	Fox Wiland ('68)	Man *Nomina Anatomica* ('68)
Vertebral	Cerebrospinal	Vertebral	Cerebrospinal	Vertebral
Spinal arterial circle	*	*	Spinal arterial circle	***
Ventral spinal	Ventral spinal	*	Arterial trunk	Anterior spinal
Dorsal spinal	*	*	*	Posterior spinal
Meningeal	*	*	*	Meningeal
Posterior inferior cerebellar	Posterior cerebellar	Posterior cerebellar	Inferior posterior cerebellar	Posterior inferior cerebellar
Basilar	Basilar	Basilar	Basilar	Basilar
Middle inferior cerebellar	*	*	*	***
Anterior inferior cerebellar	Acoustic, internal auditory	Anterior cerebellar	Inferior anterior cerebellar	Anterior inferior cerebellar
Labyrinthine	Acoustic, internal auditory	Labyrinthine	*	Labyrinthine
Accessory anterior inferior cerebellar	*	Accessory artery	*	**
Superior cerebellar	Anterior cerebellar	Superior cerebellar	Rami communicantes cranial	Superior cerebellar
Accessory superior cerebellar	*	*	*	**

* Artery not illustrated or identified in the publication.
** Term in use but has not had official adoption by the N.A.
*** Term not applicable to man.

The origin and course of the anterior inferior cerebellar and labyrinthine arteries in the dog were found to be similar to descriptions in fox, cat and man.[12–19] Confusion exists in the nomenclature of this artery in quadrupeds because of the use of the term "labyrinthine" for the artery. We found by chipping away the temporal bone that the course of the anterior inferior cerebellar artery in the dog is quite similar to its course in man, and that therefore it would appear logical that the term labyrinthine should be reserved for the artery or arteries coursing to the membranous labyrinth and the term anterior inferior cerebellar be used for the parent artery which originates from the basilar.

A number of investigators have reported the physiologic and histopathologic effects of induced macro- and micro-embolism of the labyrinthine artery in the dog and other small quadrupeds.[20–23] Investigators producing this type of embolization in the dog, however, may not have considered the contribution to the membranous labyrinth by the accessory anterior inferior cerebellar artery. This artery in our specimens was approximately one-half the diameter of the anterior inferior cerebellar and appeared capable of transporting a sizeable portion of the normal blood flow to the membranous labyrinth in the event of occlusion or ligation of the anterior inferior cerebellar artery.

Another artery of neuro-physiologic importance is a branch of the superior cerebellar which richly perfuses the upper and lateral surfaces of the caudal colliculus in the dog. The most recent *Nomina Anatomica Veterinaria* (1968) does not list this artery nor do the common anatomy textbooks of quadruped mammals. We have described the origin, course and termination of this artery (Figure 30) and suggested for it the name "artery to the caudal colliculus."[11] In other respects the superior cerebellar artery of the dog is similar to descriptions of the human superior cerebellar by Kaplan and Ford.[27]

A third previously undescribed artery in the dog[8–10] is one which we have described as arising from the basilar and coursing to the sensory root of the trigeminal nerve. It arose singly or bilaterally from the rostral portion of the basilar artery or superior cerebellar artery. After a tortuous course laterally across the pons, the artery penetrated the origin of the fifth cranial nerve between its sensory and motor roots (Figure 31). We have suggested the name "trigeminal" artery for this vessel.[11] The presence of any artery in close association with the sensory root of the trigeminal nerve makes surgical section of the pontine attachment of this nerve a hazardous procedure. The fact that in the dog there is such an artery

TABLE II.—*Portions of the brain and spinal cord supplied by the vertebral-basilar system in ten dogs*[11]

Artery	Portion of Central Nervous System Supplied
1. Dorsal spinal artery	1. Perforating branches to the lateral funiculi of the cranial segments of spinal cord
	2. Choroid plexus of fourth ventricle
2. Posterior inferior cerebellar artery	1. Perforating branches to the lateral medullary funiculi
	2. Choroid plexus of fourth ventricle
	3. Nodulus
	4. Uvula
	5. Pyramis
	6. Tuber vermis
	7. Paramedian lobule
	8. Dorsal paraflocculus
	9. Ventral paraflocculus
	10. Flocculus
	11. Ansiformis
3. Middle inferior cerebellar artery	1. Choroid plexus of fourth ventricle
	2. Medullary perforating branches
	3. Uvula
	4. Pyramis
	5. Tuber vermis
	6. Paramedian lobule
	7. Ansiformis
	8. Dorsal paraflocculus
	9. Ventral paraflocculus
	10. Flocculus
4. Anterior inferior and accessory anterior inferior cerebellar arteries	1. Medullary perforating branches
	2. Pontine perforating branches
	3. Vasa nervorum to cranial nerves V, VII and VIII
	4. Membranous labyrinth by labyrinthine arteries
	5. Choroid plexus
	6. Ventral paraflocculus
	7. Dorsal paraflocculus
	8. Cerebellar peduncles
5. Superior cerebellar artery	1. Arterial trunk
	a. Perforating branches to crus cerebri
	b. Artery to caudal colliculus
	c. Perforating branches to pontine nuclei
	2. Lateral branch
	a. Rostral portion of dorsal paraflocculus
	b. Rostral portion of ventral paraflocculus
	c. Lateral lobus simplex
	d. Ansiform lobule
	e. Medial and dorsal cerebellar peduncles
	3. Intermediate branch
	a. Medial and dorsal cerebellar peduncles
	b. Culmen
	c. Declive
	d. Lobus simplex
	e. Dorsal paraflocculus
	4. Medial branch
	—Crus cerebri
	—Brachium of caudal colliculus
	—Rostral colliculus
	Pars ascendens, medial branch
	—Central lobule
	—Ascending lobule
	—Ascending lobule
	—Culmen
	—Paramedian lobule
	—Declive
	—Folium
	—Lobus simplex
	—Dorsal portion of ansiformis
	—Tuber vermis
	Pars descendens, medial branch
	—Lingual
	—Central lobule
	—Ascending lobule
	—Culmen

makes the dog an interesting potential experimental model for the study of diseases of the trigeminal nerve, as aberrant arteries have been described in the region of the sensory root of the trigeminal nerve in man and their accidental section during surgery of the sensory root has resulted in fatal hemorrhage.[13]

Although the basilar artery of man generally arises from the vertebral arteries at the lower border of the pons, more rostrally than in the dog, the branches of the basilar artery are similar in many respects in the two species. With the exception of the middle inferior cerebellar artery not listed in *Nomina Anatomica* all other branches of the basilar artery of man may be found in the dog.

BLOOD FLOW MEASUREMENTS IN THE BASILAR ARTERY

Following the study of the anatomy of the vertebral-basilar system in the dog, an intermandibular-transclival approach to the posterior cranial fossa was developed which allowed exposure of the basilar artery for attachment of a small electromagnetic blood flow transducer (Figure 32) and basilar blood flow was measured in this manner in 15 experiments.[24] The results of single intravenous injections of betahistine hydrochloride indicate a mean increase in basilar artery blood flow of 54% and a simultaneous decrease in systemic arterial blood pressure of a duration of action of approxi-

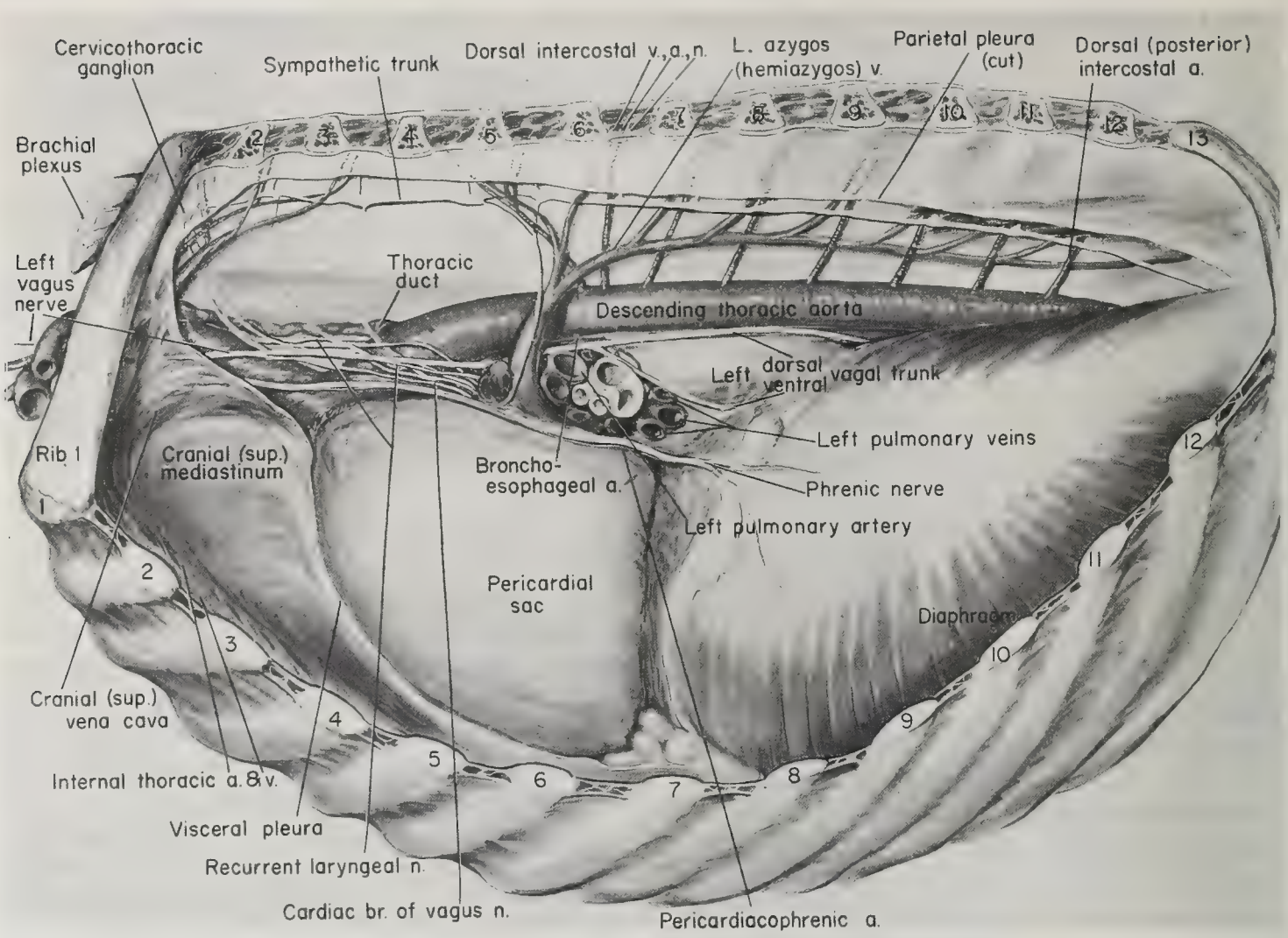


FIGURE 24.—Left mediastinum illustrating the azygos venous system.

mately one minute. Histamine phosphate yields results similar to betahistine hydrochloride, while nicotinic acid produces only slight increases in blood flow in the basilar artery (Figure 33).

ARTERIAL SUPPLY AND AUTONOMIC INNERVATION OF THE STOMACH OF SHEEP AND CALF

The ruminant animal has three developmental dilatations of the lower esophagus, namely the reticulum, omasum and rumen, and they are collectively referred to as the forestomach. The true stomach, comparable to the stomach in man, is called the abomasum in ruminant mammals. Because of the increasing interest in the study of physiology of digestion and its relation to the cardiovascular system, a study of the normal arterial supply to the ruminant stomach was undertaken.[25,26]

Based on a study of ten adult sheep, the arterial supply to the ovine stomach is derived from the celiac artery. Cranial mesenteric and celiac arteries originate by a common trunk from the abdominal aorta in most cases. The common hepatic artery (Figure 34), carrying with it a dense plexus of autonomic nerves, courses through the lesser omentum to a position near the porta hepatis where it splits into right and left hepatic, cystic, right gastric, and gastroduodenal arteries. The gastroduodenal artery contributes blood supply to the descending duodenum and then courses to the greater curvature of the abomasum. The left gastric artery (Figure 35) and its large gastroepiploic branch are the major arteries supplying the abomasal fundus and body. The left gastric artery anas-

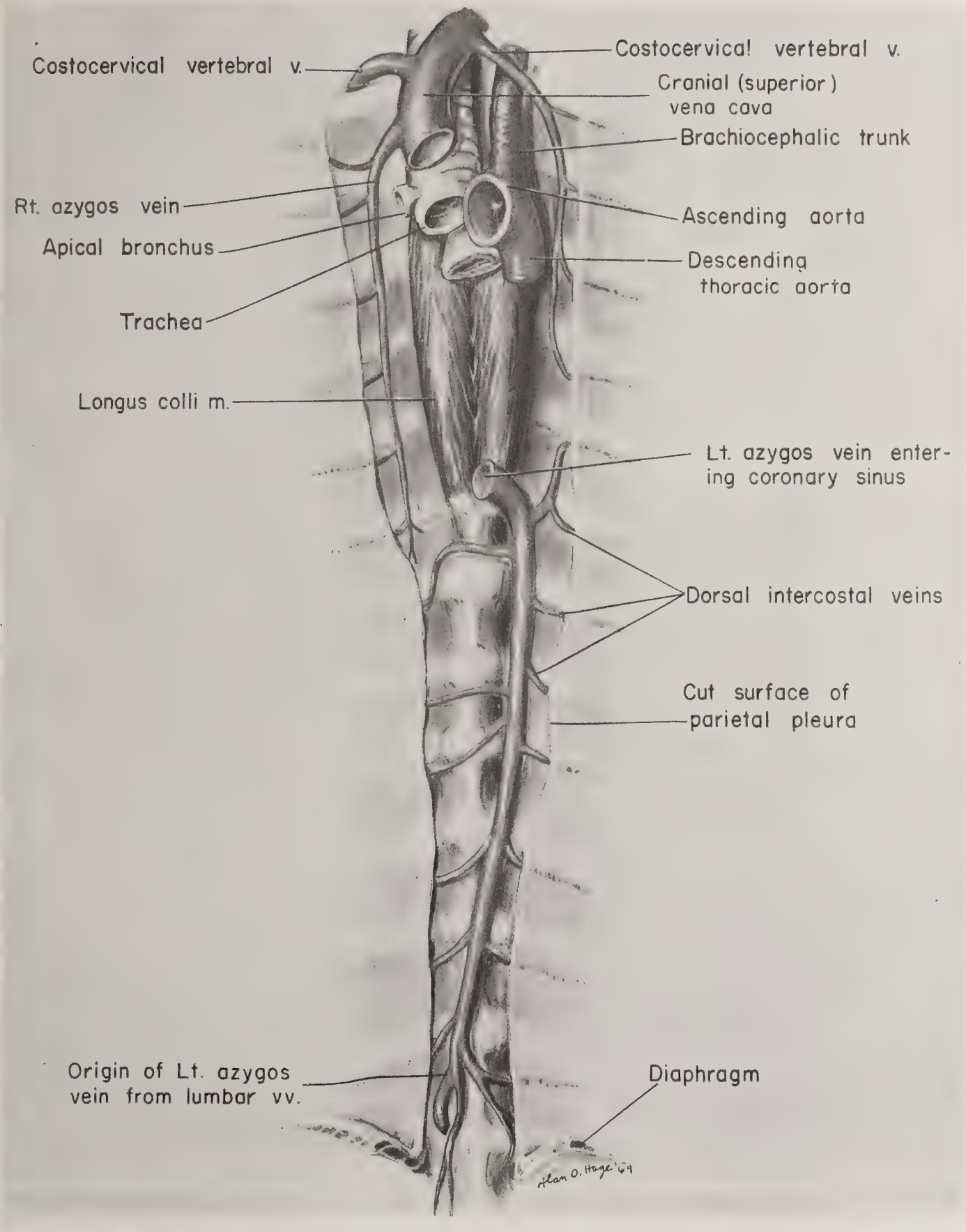


FIGURE 25.—Azygos system of veins.

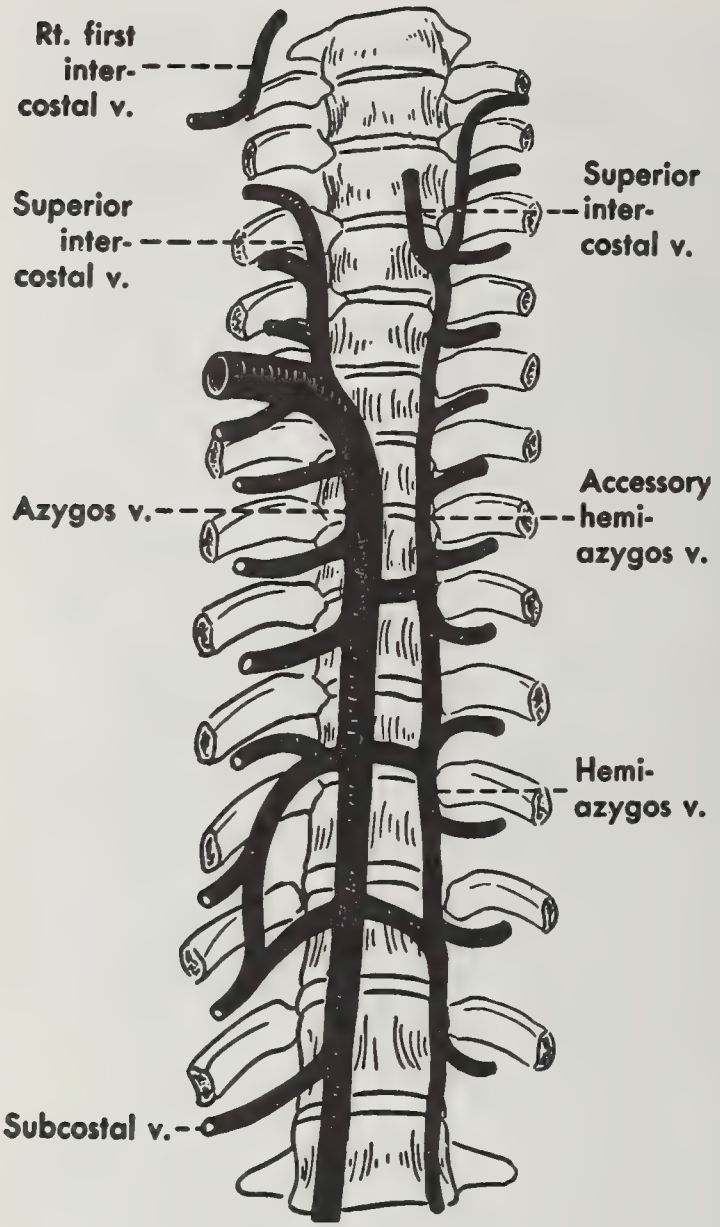


FIGURE 26.—The azygos system of veins. In this example there are good connections between the various parts of the hemiazygos system, annd several connections to the azygos (man) vein.

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

tomoses on the abomasum with the right gastric artery, a branch of the common hepatic artery, whereas the left gastroepiploic artery anastomoses along the greater curvature with the right gastroepiploic artery, a branch of the gastroduodenal artery, likewise from the common hepatic artery (Figure 34). The splenic artery in the ruminant animal is smaller than that in the monogastric animal. It arises from the celiac artery by a common trunk with the right ruminal artery (Figure 36), and an accessory artery to the spleen often originates from the left ruminal artery (Figure 37). The reticular artery (Figures 37 and 38) is the largest branch of the left ruminal artery before the latter dips into the left longitudinal groove. The reticular artery supplies the distal part of the esophagus, the area of the cardia, and the atrium ventriculi, and it sends grossly visible arterial twigs as vasa nervorum through the esophageal hiatus to the dorsal and ventral vagal trunks. There is the possibility of infarction of visceral structures supplied by the descending portion of the reticular artery should the artery be obstructed by ligation in surgical procedures or by thrombosis. The gastric (esophageal) groove receives many arterial twigs from the left gastroepiploic branch of the left gastric artery. The ruminant stomach receives parasympathetic innervation by the dorsal and ventral vagal trunks (Figure 39). The ventral vagus nerve innervates the diaphragmatic surface of the reticulum, the area of the atrium ventriculi liver and gall bladder, and fibers (the long pyloric nerve) followed the right gastric artery to the lesser curvature of the true stomach (abomasum). The dorsal vagus nerve courses to the visceral surface of the reticulum and sends branches along the right ruminal and left gastric arteries to the dorsal and ventral sacs of the rumen and both curvatures of the abomasum. The dorsal vagus also contributes to the celiac and cranial mesenteric plexuses.

The sympathetic innervation of the ruminant stomach is derived from the celiac, and cranial mesenteric plexuses and branches from the plexuses course to the stomach with arteries originating from the celiac trunk.

In surgical procedures involving removal of the rumen and reticulum, the major vessels which must be identified and ligated are the right and left ruminal arteries, while blood vessels which must be preserved in order to avoid infarction of the true stomach are the common hepatic, left gastric and splenic vessels.



FIGURE 27.—Plastic cast of the arterial supply to the cervical segments of the spinal cord of sheep. Pointer indicates a radicular branch of a spinal artery originating from the vertebral artery.



FIGURE 28.—Arterial supply to thoracic segments of the spinal cord of the sheep. A sponge (ep.) has been placed over the ventral spinal artery to simulate the position of the spinal cord. Dorsal intercostal arteries (dor. int. arteries) give off spinal branches which enter the intervertebral foramina to reinforce the tiny ventral spinal artery. The left bronchial artery is visible at lower portion of the figure.

THE FEMORAL TRIANGLE AND COLLATERAL CIRCULATION TO THE CAUDAL EXTREMITY

The Femoral Triangle

After the fascia lata over the front of the thigh has been removed, the femoral triangle and its contents are visible. In the sheep the sartorius muscle covers the contents of the triangle (Figure 40) and makes vena puncture or arterial puncture somewhat of a different experience than in the dog or man where the triangle is bounded laterally by the sartorius and medially by the pectineus muscles (Figure 41). Within the triangles the contents are the femoral vein most medial and femoral artery intermediate between the femoral nerve or saphenous branch of the femoral nerve.

Collateral Circulation to the Distal Pelvic Limb

The well developed profunda femoris artery in the dog lies partly within and partly outside of the abdominal cavity. It establishes an extensive collateral circulation with the lower portion of the femoral artery by way of anastomoses with the cranial femoral, muscular branches of the femoral and lower down with the caudal femoral artery (Figure 42). This unusual collateral arterial circulation to the pelvic limb in the dog enables one to ligate the femoral artery for purpose of catheterizing the external iliac artery or abdominal aorta. In a series of

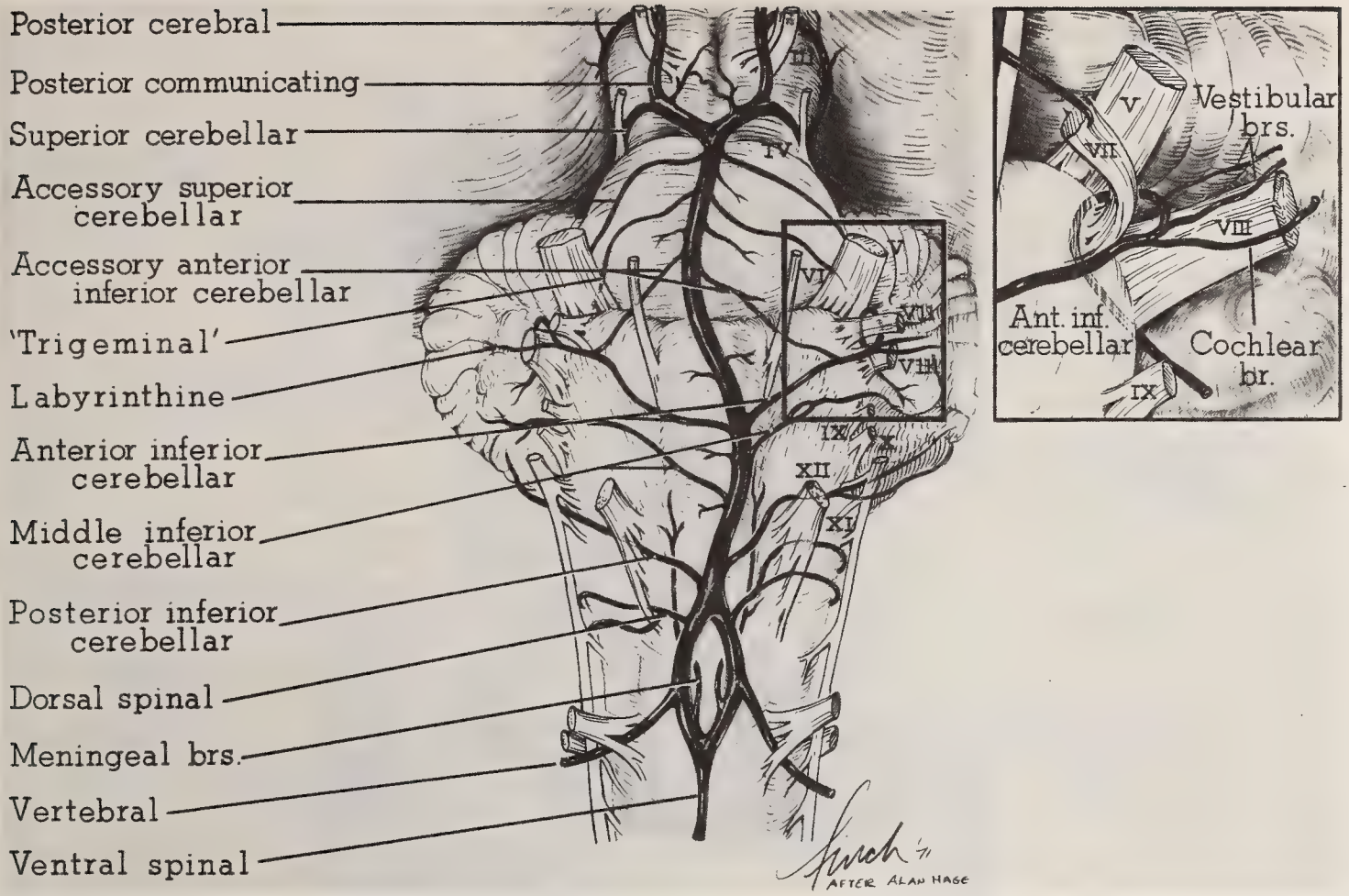


FIGURE 29.—Common pattern of the basilar artery of the dog from 30 specimens. Variations in arterial relations in the porus acusticus and internal acoustic meatus are shown (insert, upper right).

35 experiments studying hypovolemic shock,[28] it was necessary to bilaterally ligate the femoral artery in dogs for purposes of blood pressure measurements and withdrawing blood into a shed-blood reservoir. All animals maintained adequate blood flow to the distal limbs and sustained no ischemic complications from the femoral ligations.

Collateral circulation to the distal pelvic limb of the horse (Figure 43) is not as well developed as is the dog's; however it appears to be much better than the collateral circulation in the pelvic limb of the calf (Figure 44).

TABLE III.—*Key to the Major Arteries of the Pelvic Limb of the Dog, Calf and Horse*

1. Aorta	5. Femoral
2. Deep circumflex iliac	5.1 Cranial femoral
3. Internal iliac	5.2 Descending geniculate
3.1 Cranial gluteal	5.3 Caudal femoral
3.2 Caudal gluteal	5.4 Saphenous
4. External iliac	6. Popliteal
4.1 Pudendoepigastric trunk	6.1 Caudal tibial
4.2 Deep femoral	6.2 Cranial tibial
4.3 Obturator artery	

SUMMARY

Aspects of the gross and microscopic anatomy of the cardiovascular systems of the sheep, calf, dog, pig and horse were correlated with the cardiovascular system of man. They were:

(1) thorax;
(2) arterial supply to the spinal cord;
(3) the vertebral-basilar system of the brain;
(4) blood flow measurements in the basilar artery;
(5) arterial supply and autonomic innervation of the stomach in sheep and calf; and
(6) femoral triangle and collateral circulation to the caudal extremities.

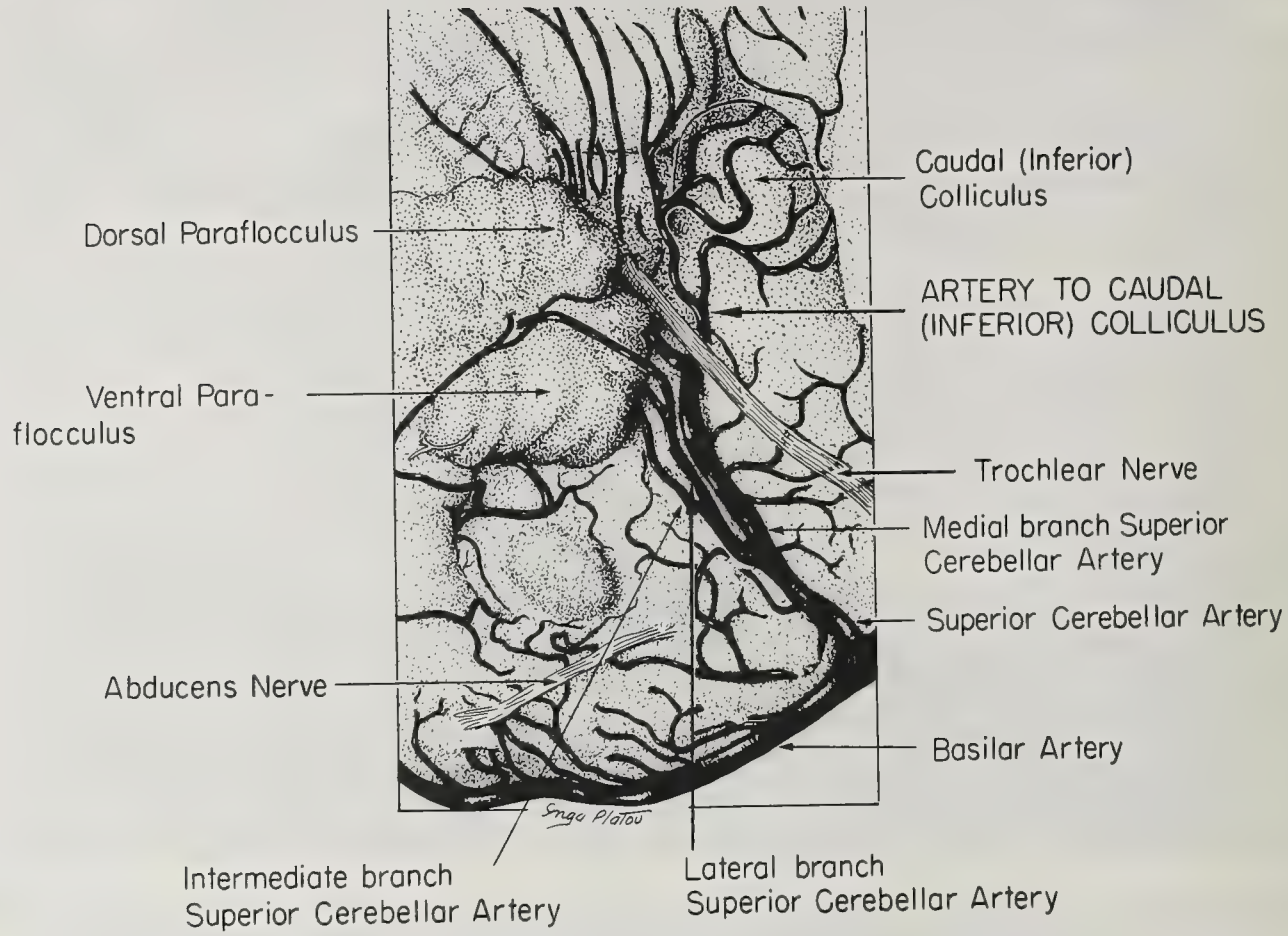


FIGURE 30.—Branches of the superior cerebellar artery. After arising from the basilar artery, the superior cerebellar artery trifurcated into medial, lateral and intermediate branches. The medial branch gave origin to the artery of the caudal colliculus.

REFERENCES

1. ANDERSON, W. D. *Thorax of Sheep and Man.* Dillon Press, Minneapolis, 1971.
2. ANSON, B. J., ed. *Morris' Human Anatomy;* 12th ed., The Blakiston Division, McGraw-Hill, New York/Toronto/Sydney/London, p. 635, 1966.
3. CAULDWELL, E. W., SIEKERT, R. G., LININGER, R. E., and ANSON, B. J. The Bronchial Arteries. Surg. Gynec. & Obst. 86:395–412, 1948.
4. HENSON, R. A., and PARSONS, M. Ischemic lesions of the spinal cord: An illustrated review. Quart J. Med. 142:205–222, 1967.
5. BURTON, C. Spontaneous ischemic infarction of the spinal cord with traumatic sequela. Stroke 1:397–400, 1970.
6. RITTMAN, W. W., and SMITH, L. L. Cerebral flow following severe hemorrhage. Surg. Gyn., Obstet. 123:67–72, 1966.
7. SUNDERLAND, S. The arterial relations of labyrinth of the ear of dog and of man. Am. J. Anat. 32:189–193, 1945.
8. MILLER, M. E., CHRISTENSEN, G. C., and EVANS, H. E. *Anatomy of the Dog,* W. B. Saunders, Philadelphia, pp. 311–315, 1964.
9. POPESKO, P. *Atlas of Topographical and Applied Anatomy of the Domestic Animals,* R. Getty, ed. W. B. Saunders, Philadelphia, 1970.
10. HOLLINSHEAD, W. H. *Textbook of Anatomy,* 2nd ed. Hoeber, Harper & Row, 1967.
11. ANDERSON, W. D., and KUBICEK, W. The vertebral-

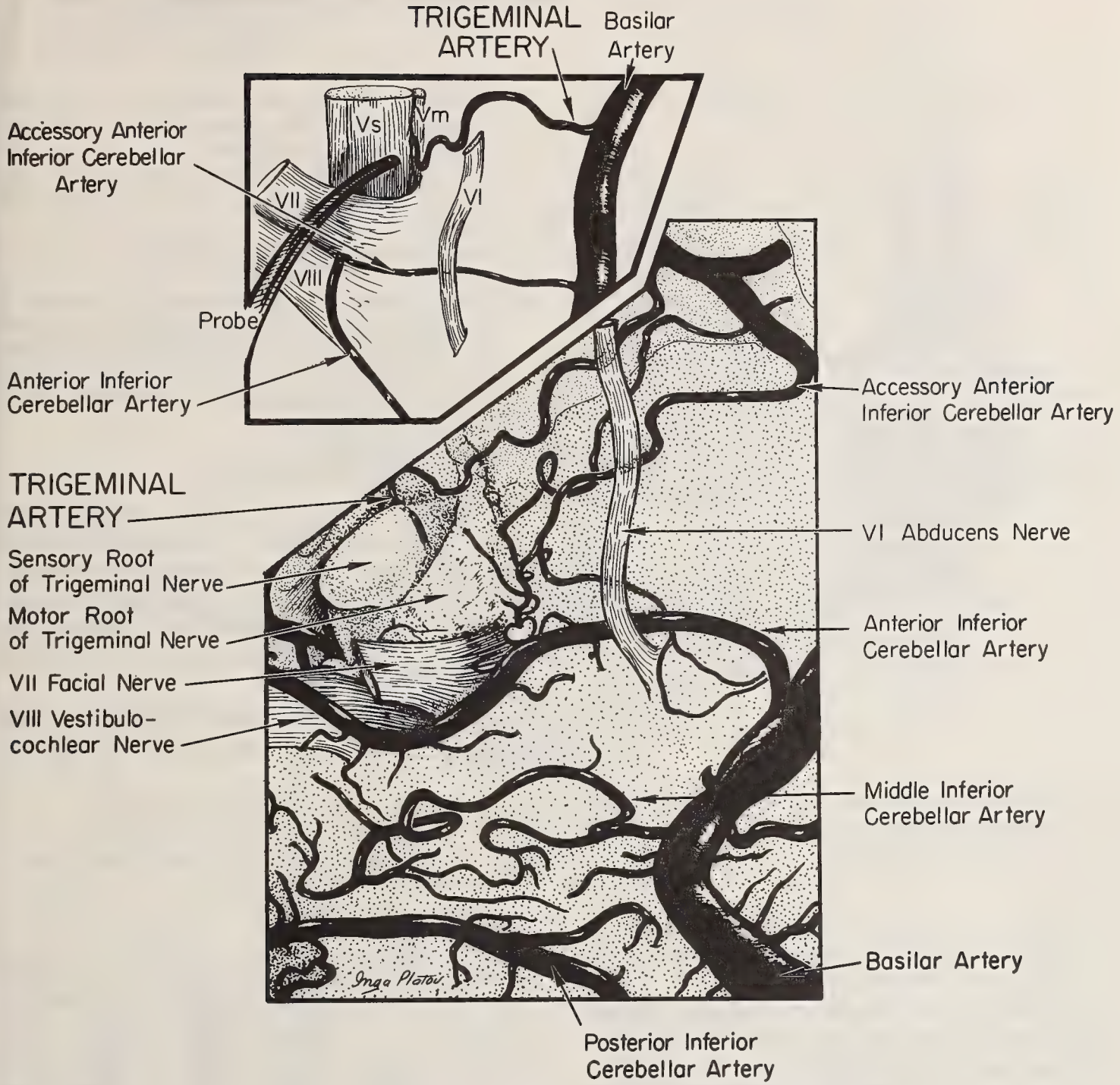


FIGURE 31.—Trigeminal artery of the dog coursing across the pons and penetrating the brain between the sensory and motor roots of the trigeminal nerve.

basilar system of dog in relation to man and other mammals. Am. J. Anat. 132:179–188, 1971.

12. WILAND, C. The basilar artery (a. basilaris cerebri) of the brain in foxes. Folia. Morph. (Warsz) 25: 645–649, 1966.
13. WATT, J. C., and MCKILLOP, A. N. Relation of arteries to roots of nerves in posterior cranial fossa in man. Arch. Surg. 30:336–345, 1935.
14. FERRARI-LELLI, G. Comparamento dell' arteria uditiva interna e dei suoi rami labirintici nell'uomo. Z. Anat. Entwicklungsgesch. 110:48–80, 1939.
15. SUNDERLAND, S. The arterial relations of the internal auditory meatus. Brain 68:23–27, 1945.
16. KIMURA, R., and PERLMAN, H. B. Arterial obstruction of the labyrinth. Ann. Oto. Rhinol. Laryng. 67:5–24, 1958.
17. KELLNER, F., and RICHTER, O. B. On the development of the blood vessels of the internal ear in man. Mschr. Ohrenheilk. 96:187–209, 1962.

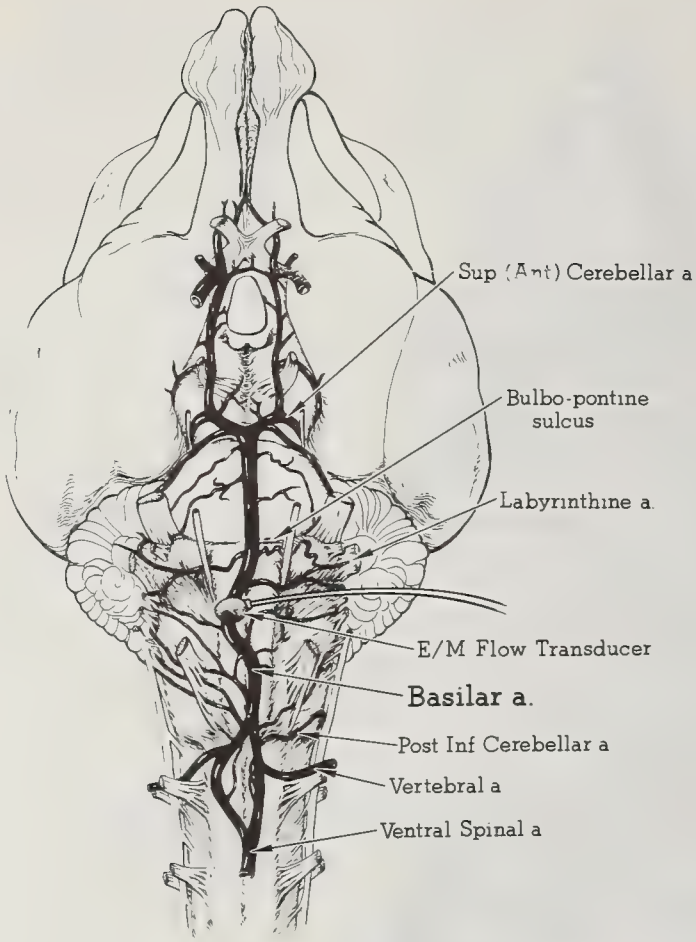


FIGURE 32.—Site of attachment of electromagnetic blood flow transducer (1 mm lumen diameter) for measurement of basilar blood flow.

18. MAZZONI, A. Internal auditory canal arterial relations at the porus acusticus. Ann. Oto. Rhinol. Laryng. 78:1–18, 1969.
19. MAZZONI, A., and HANSEN, C. C. Surgical anatomy of the arteries of the internal auditory canal. Arch. Otolaryng. 91:128–135, 1969.
20. ALFORD, B. R., SHAVER, E. F., ROSENBERG, J. J., and GUILFORD, F. R. Physiologic and histopathologic effects of microembolism of the internal auditory artery. Trans. Am. Otal. Soc. 53:110–130, 1965.
21. BERNSTEIN, J. M., and SILVERSTEIN, M. Anterior cerebellar and labyrinthine arteries. A study in the cat, Arch. Otolaryng. 83:402–435, 1966.
22. PERLMAN, H. B., KIMURA, R., and FERNANDES, C. Experiments on temporary obstruction of the internal auditory artery. Laryngoscope 69:591–600, 1959.
23. HILGER, J. A. Vasomotor labyrinthine ischemia, Ann. Oto. Rhinol. Laryng. 59:1102–1108, 1950.
24. ANDERSON, W. D., and KUBICEK, W. Effects of betahistine HC1, nicotinic acid, and histamine on basilar blood flow in anesthetized dogs. Stroke 2: 409–415, 1971.
25. ANDERSON, W. D., and WEBER, A. F. Normal arterial supply to the ruminant (ovine) stomach. J. Anim. Sci. 28:379–382, 1969.
26. ANDERSON, W. D. Surgical anatomy associated with rumenoreticulectomy in the sheep. Am. J. Vet. Res. 30:1631–1636, 1969.
27. KAPLAN, H. A. and FORD, D. H. *The Brain Vascular System*, New York, N.Y., Elsevier.
28. KUBICEK, W., and ANDERSON, W. D. Effects of betahistine HC1 (Serc) on recovery from induced hypovolemic shock in dogs, VI European Conference on Microcirculation, Proceedings, Aalborg, Denmark, S. Karger, Basel (Switzerland), 160–163, 1971.

DISCUSSION

CHAIRMAN KUSSEROW: Thank you, Dr. Anderson. As you stressed, for those concerned with the research area of prosthetic vascular implants, the differences in anatomical structure of the vascular system in the various species is a very important matter. Are there questions?

JEFFREY L. PETERS, University of Utah, Salt Lake City: Let's say one blocks off the left

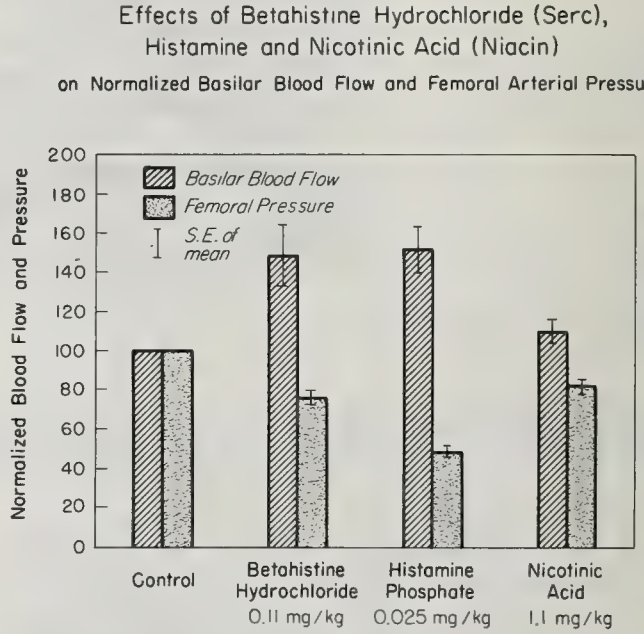


FIGURE 33.—Mean changes (± SEM) in basilar blood flow in six experiments in anesthetized dog following intravenous injection of betahistine hydrochloride (Serc), histamine phosphate and nictotinic acid (Niacin).

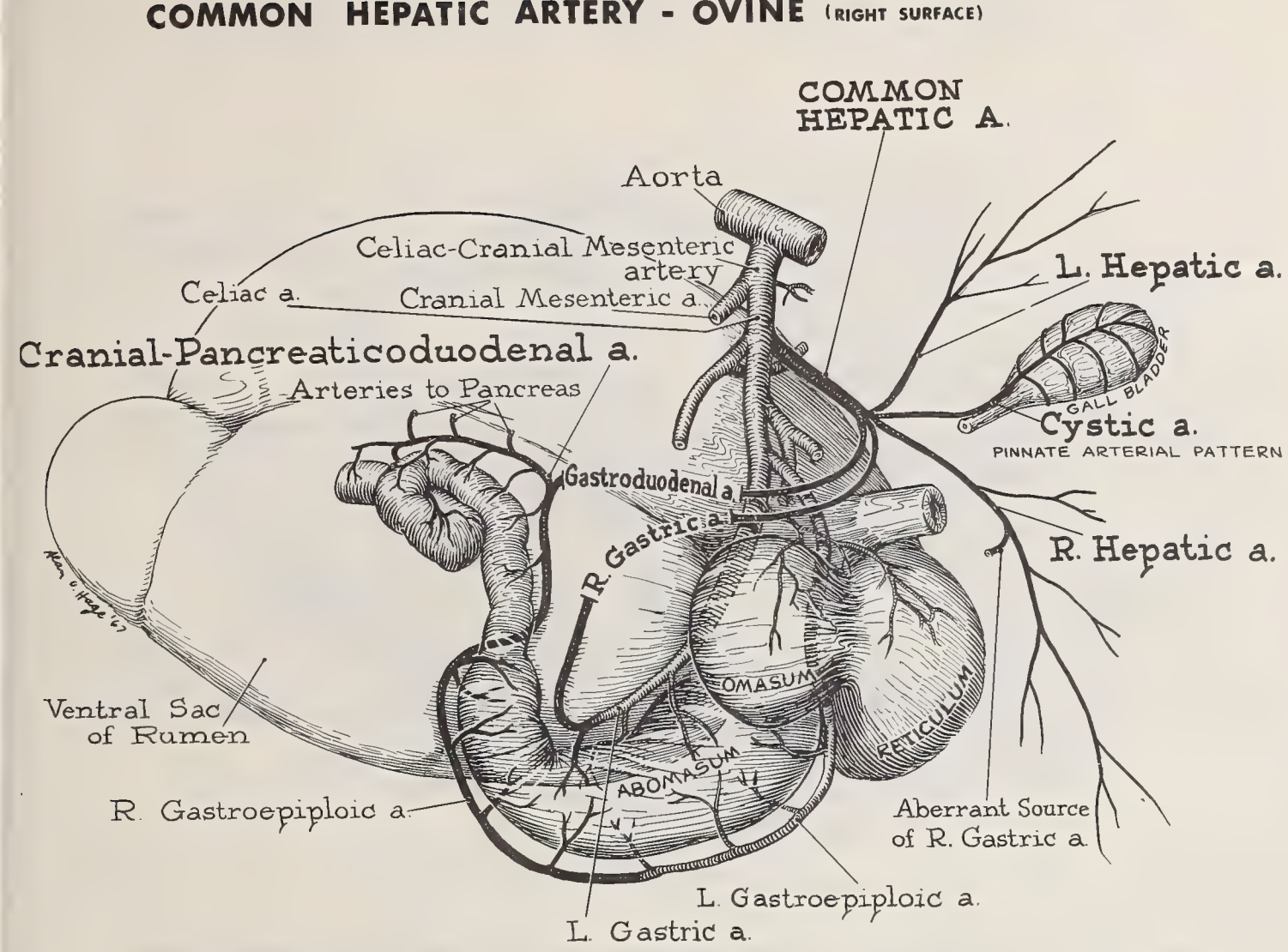


FIGURE 34.—Common hepatic artery of sheep (right surface).

azygos vein in the bovine. What is the collateral circulation for that vein? Would you predict the possibility of hydrothorax if one does this?

DR. ANDERSON: Well, the left azygos or the hemi-azygos, you know, is the dominant venous drainage. There are intracommunications with the azygos or the right azygos. I would fear, perhaps, the consequences. However, many of these collaterals do have a way of opening up with time, if the ligation is not complete. However, we must be careful about dealing with the left azygos or the hemi-azygos in sheep and calf and pig.

FREDERICK SPERLING, Howard University, Washington, D.C.: Could you please elaborate on the statement you made with regard to ligating the brachial artery leading to pulmonary disease?

DR. ANDERSON: When we first began this work in 1969, we were told by the people in the artificial heart program that they were having problems with respiratory disease following the ligation of the brachial artery. They were not properly identifying this. Later on, with further dissections, they found that they had been disrupting, destroying, or ligating the brachial artery. There's some work going on at the Veterans' Hospitals throughout the United States (I believe Minnesota is one place), in which the following theory has been proposed: that the segment of aorta from which the two or three brachial arteries arise should also be

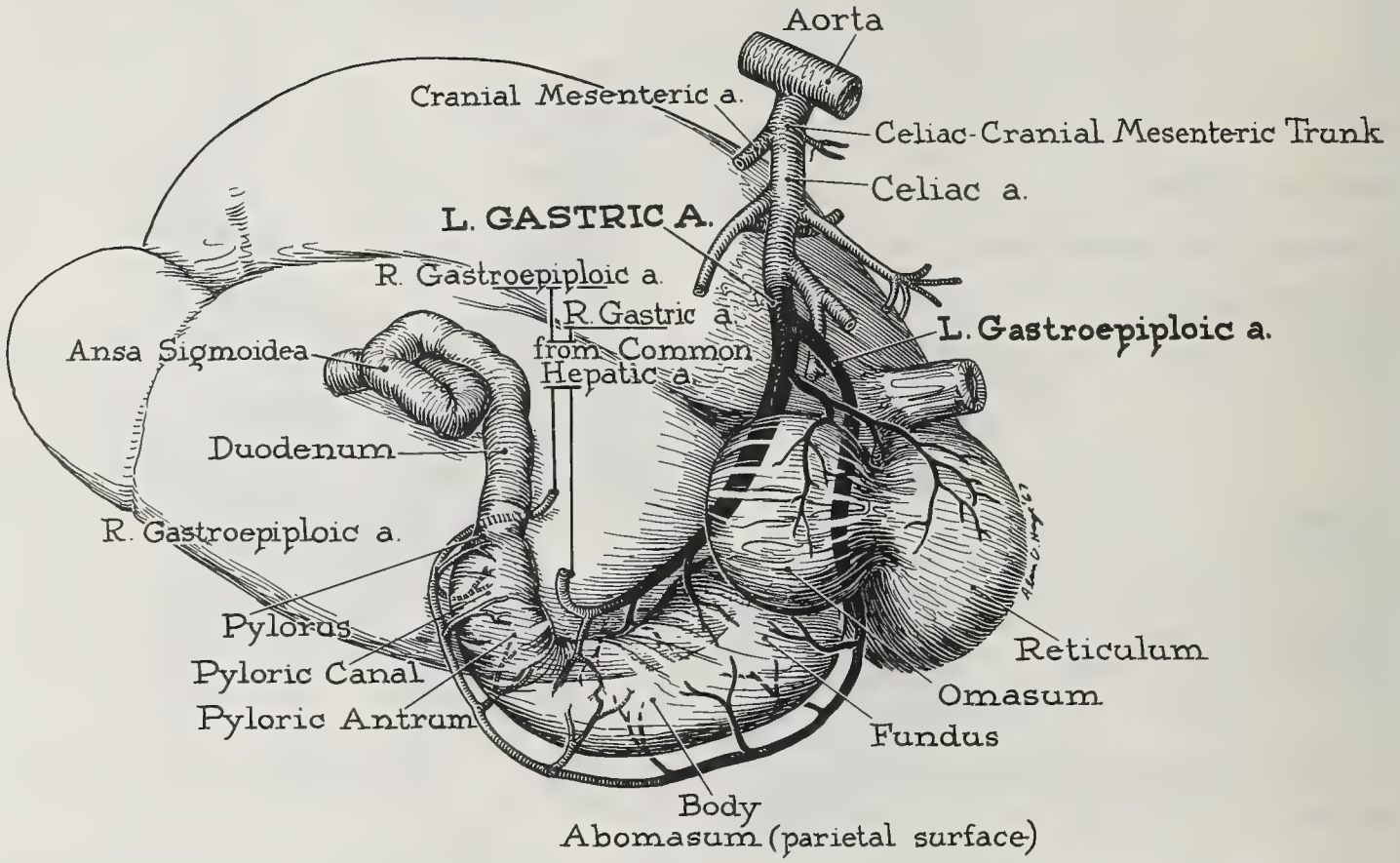


FIGURE 35.—Left gastric artery of sheep (right surface). Point of ligation of reticular branch.

transplanted with the transplanted donor lung. Certainly this is another area that is very hard to dissect. About the only way we can demonstrate and document it fairly accurately is with the plastic casts. Another area where attention should be devoted concerning the consequences of total ablation is the brachial arteries.

NORMAN B. GUILLOUD, Squibb Institute, New Brunswick, N.J.: I wonder if there is a publication which you can cite that adequately compares the anatomy of domestic and/or laboratory animals with that of man?

DR. ANDERSON: There is one by W. D. Anderson that just came out. It's called "The Thorax in Sheep and Man," and it's published by Dillon Press, Minneapolis.

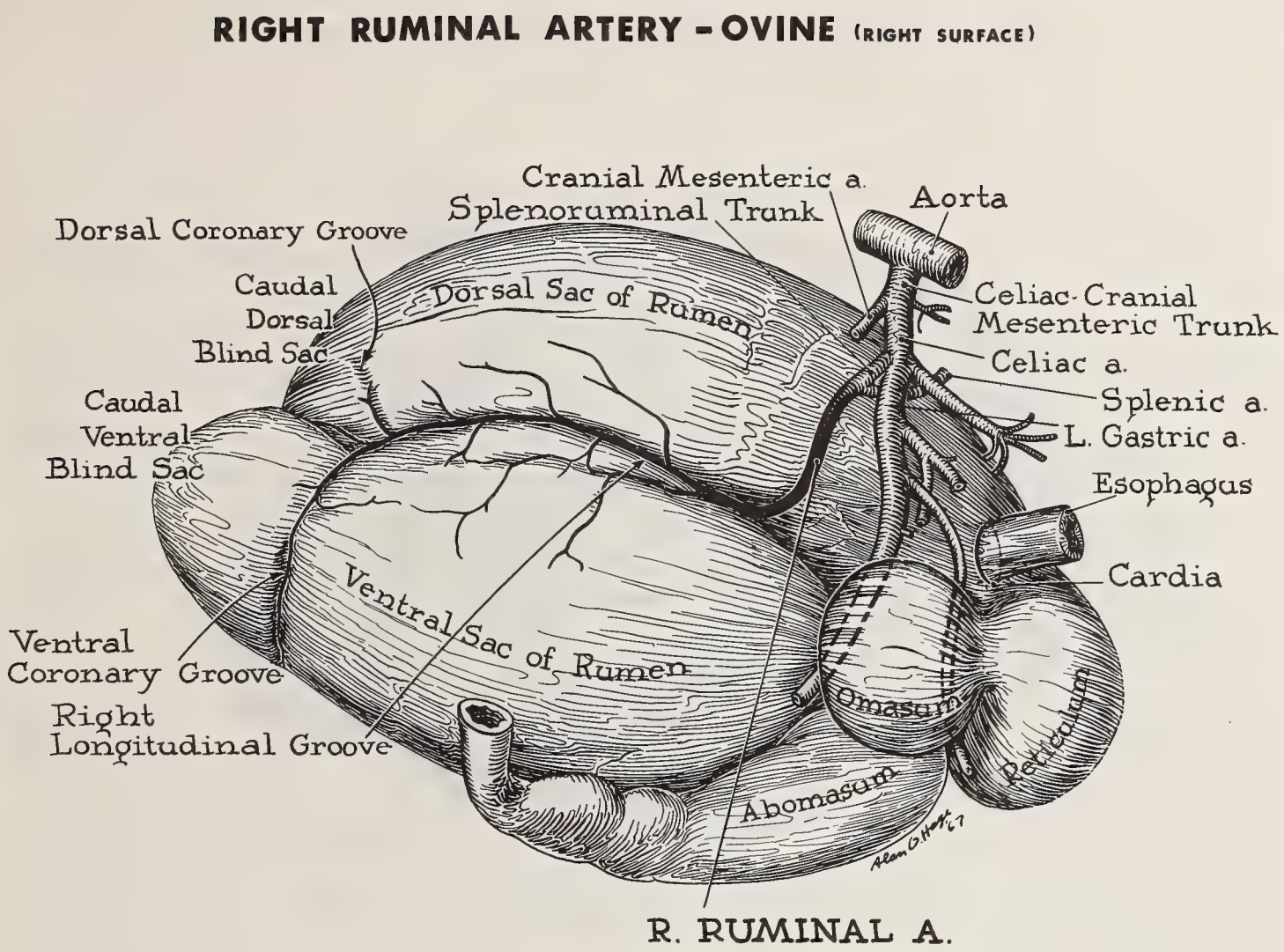


FIGURE 36.—Right ruminal artery of sheep (right surface).

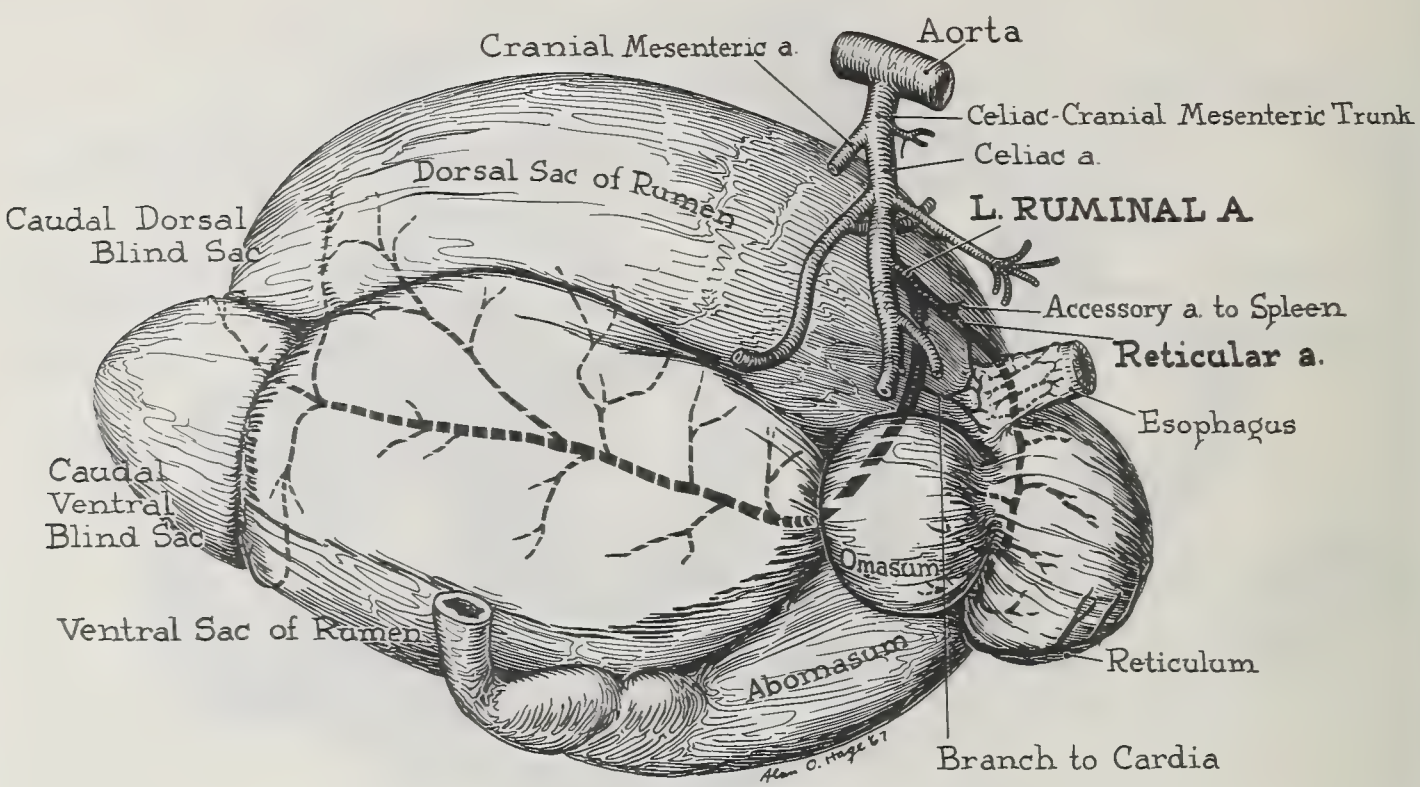


FIGURE 37.—Left ruminal artery of sheep (right surface).

LEFT RUMINAL ARTERY - OVINE (LEFT SURFACE)

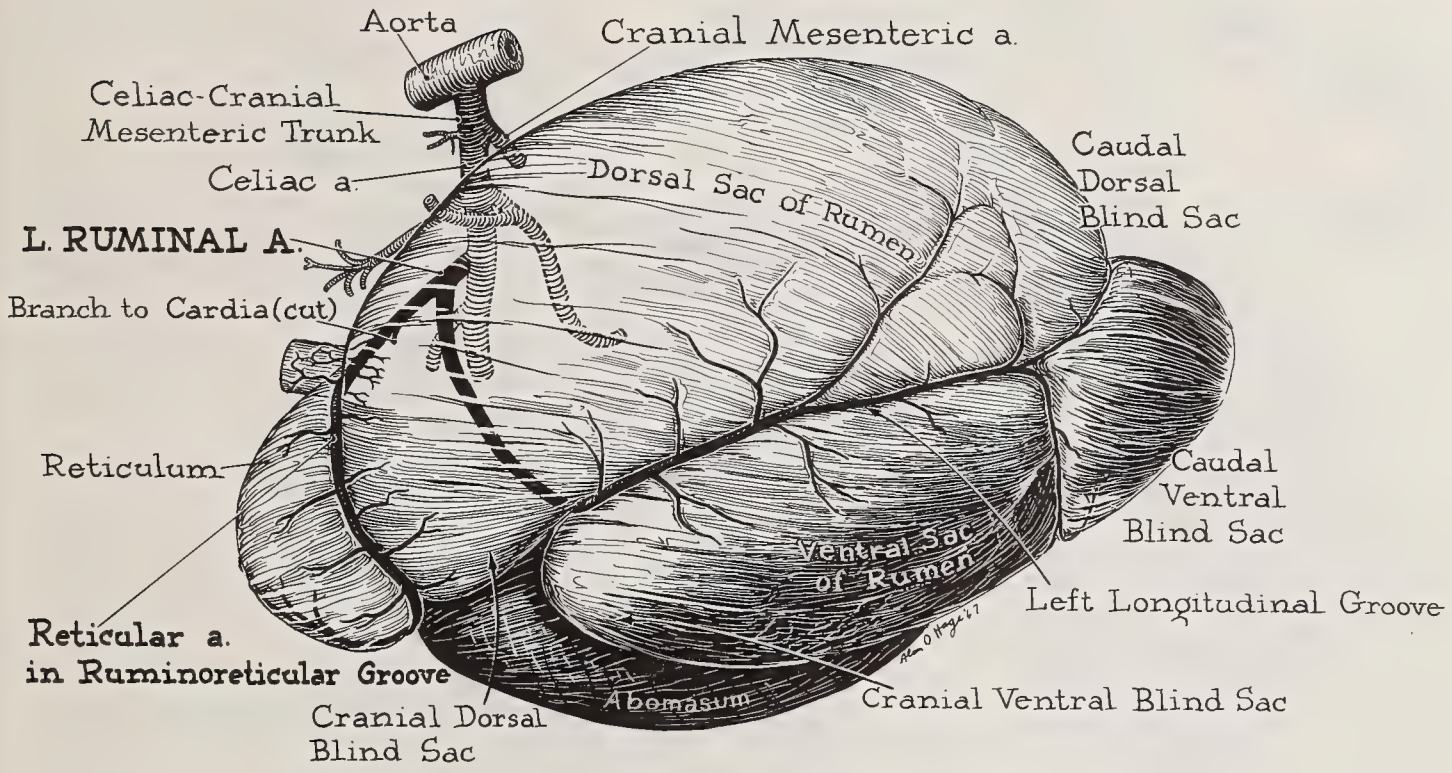
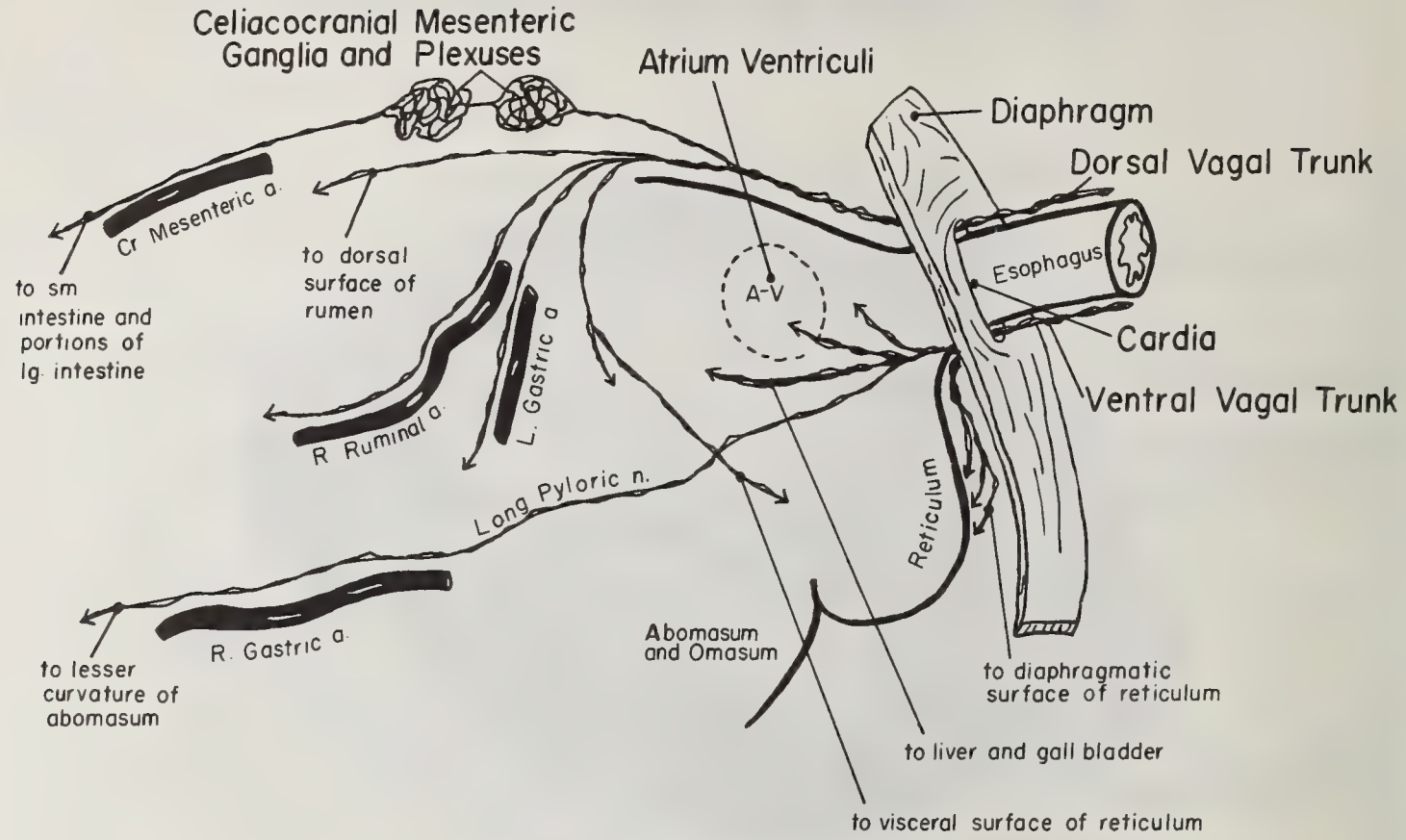


FIGURE 38.—Left ruminal artery of sheep (left surface).



TERMINATIONS OF VAGAL TRUNKS IN RUMINANT

adapted from Habel, R.E., 1964, Guide to the Dissection of Domestic Ruminants, Ann Arbor: Edwards Bros.

FIGURE 39.—Terminations of vagal trunks in sheep.

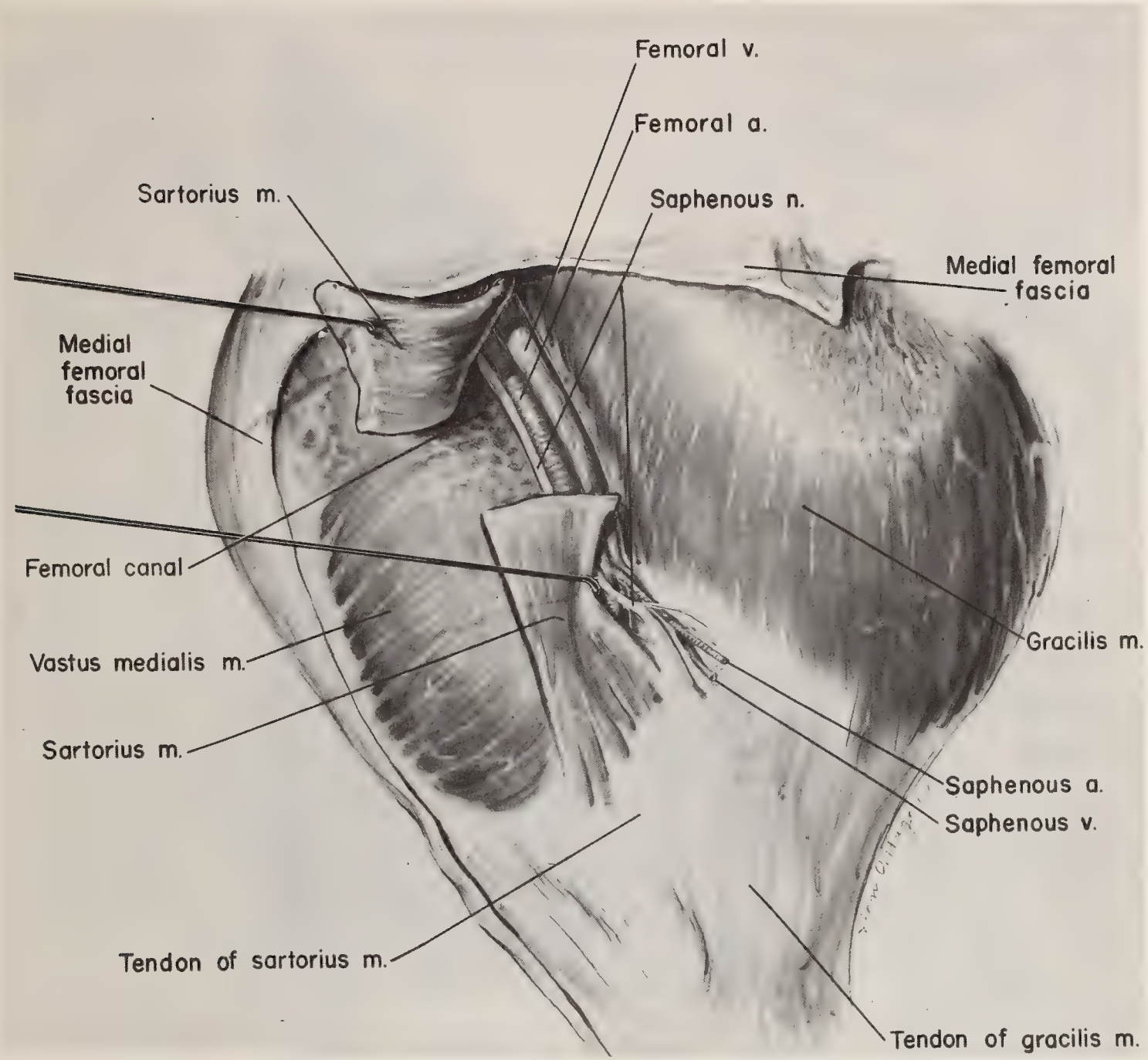


FIGURE 40.—Femoral triangle and contents (sheep).

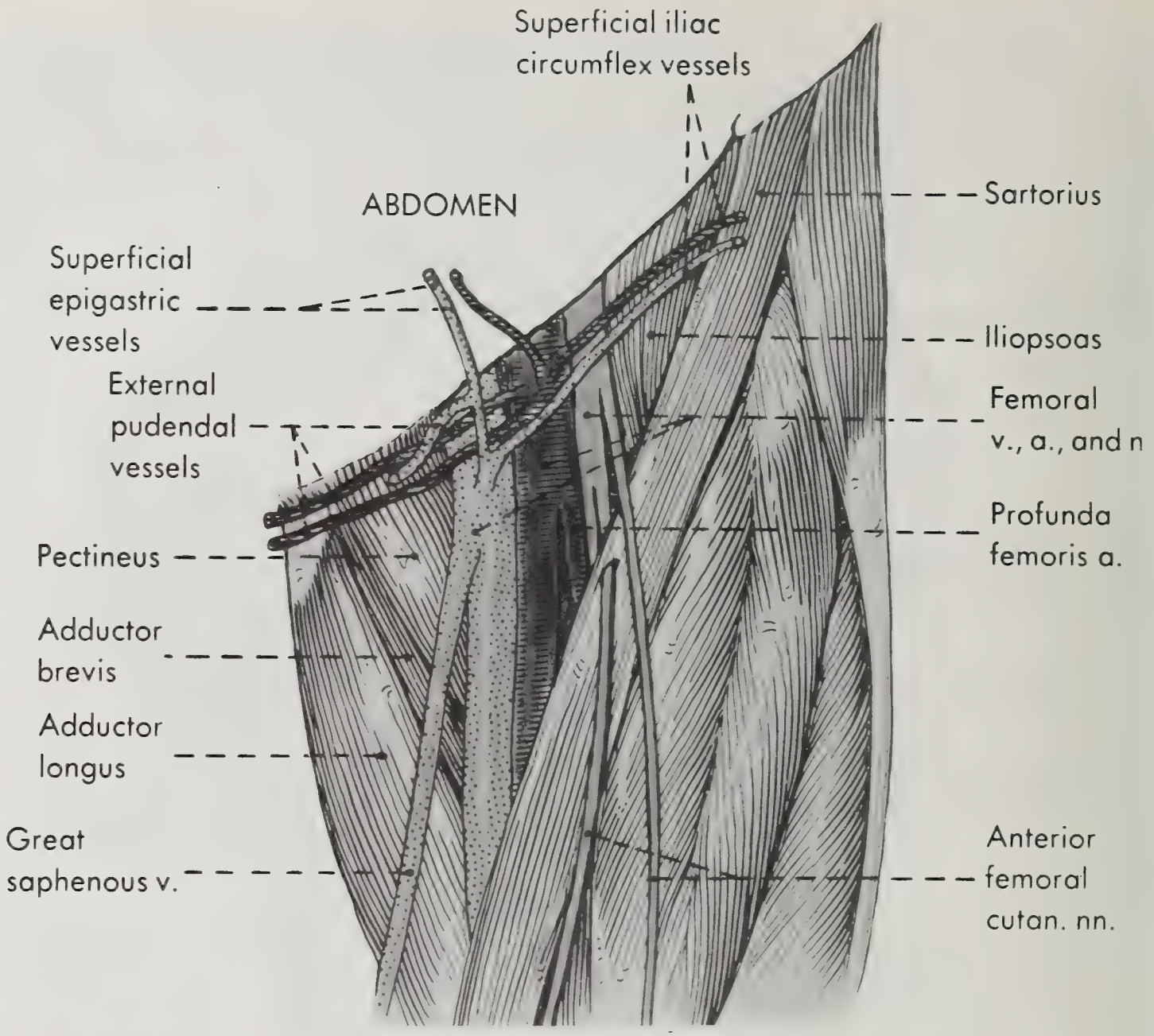


FIGURE 41.—Femoral triangle and contents (man).

Reproduced by permission from: W. Henry Hollinshead, TEXTBOOK OF ANATOMY (ed. 2). Copyright (1967). Hoeber Medical Division—Harper & Row., New York.

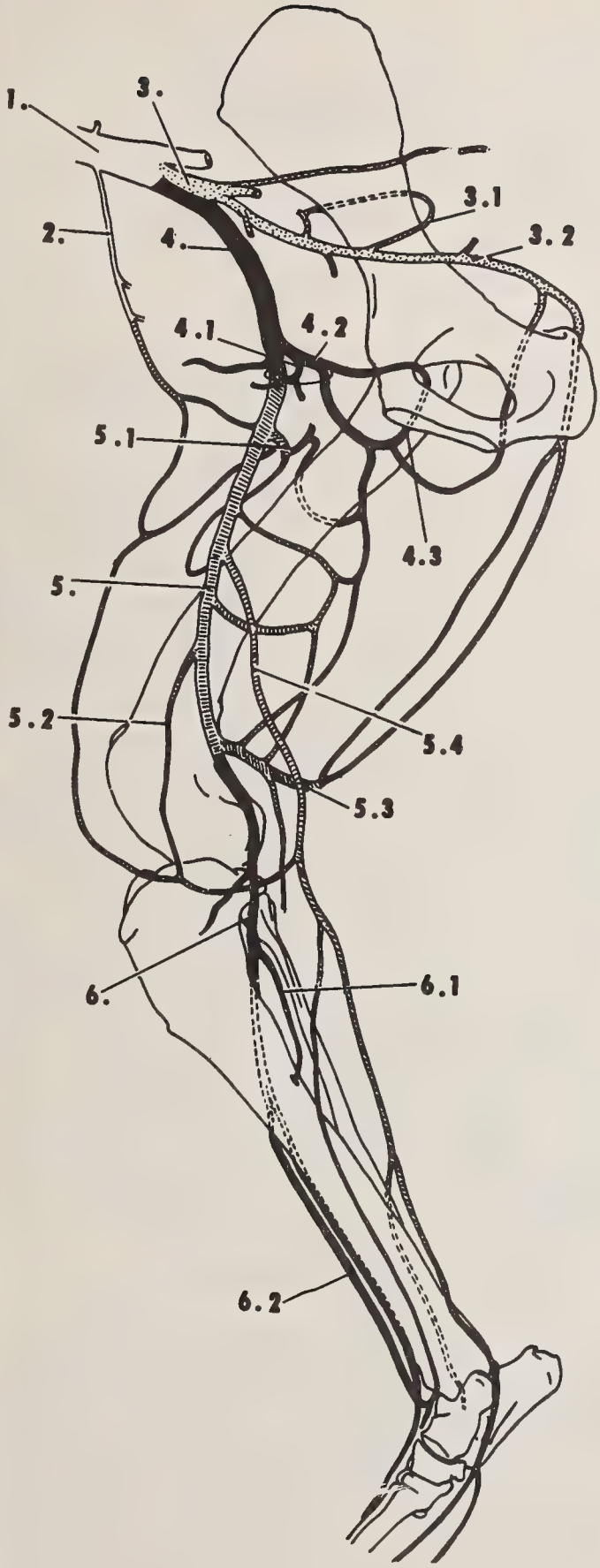


FIGURE 42.—Major arteries of pelvic limb of dog.

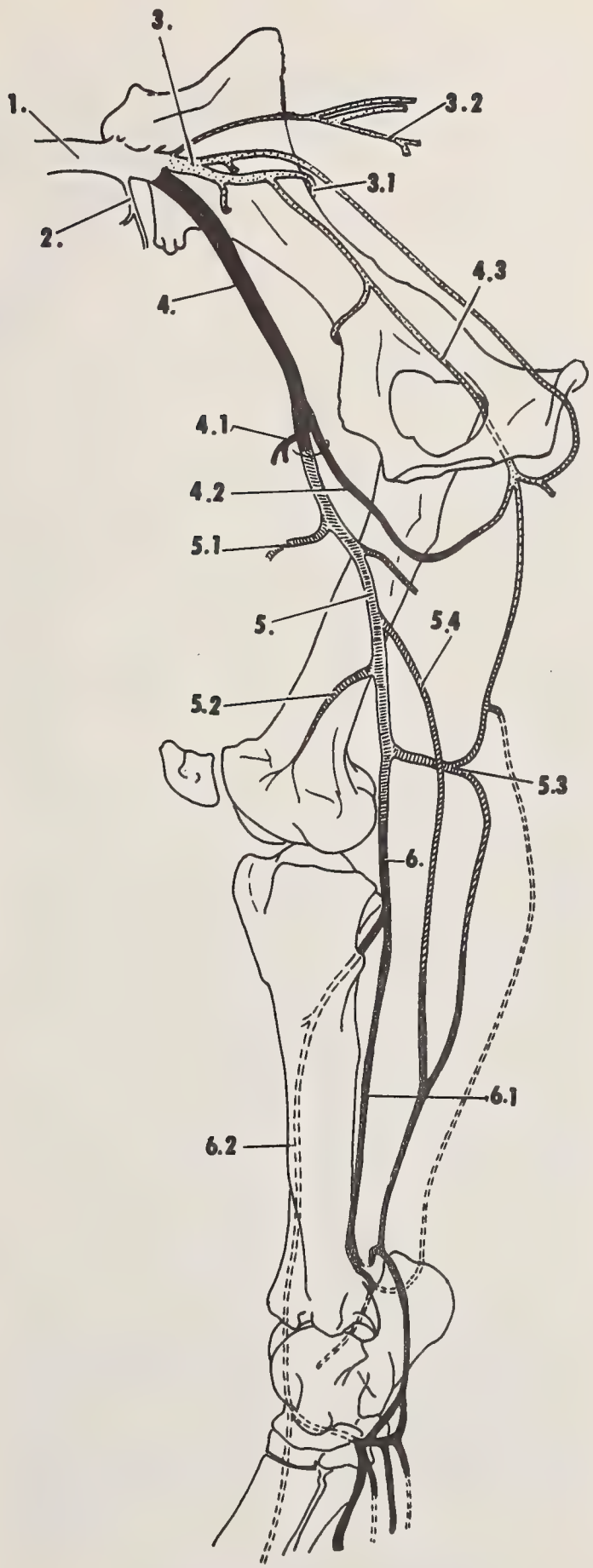


FIGURE 43.—Major arteries to pelvic limb of horse.

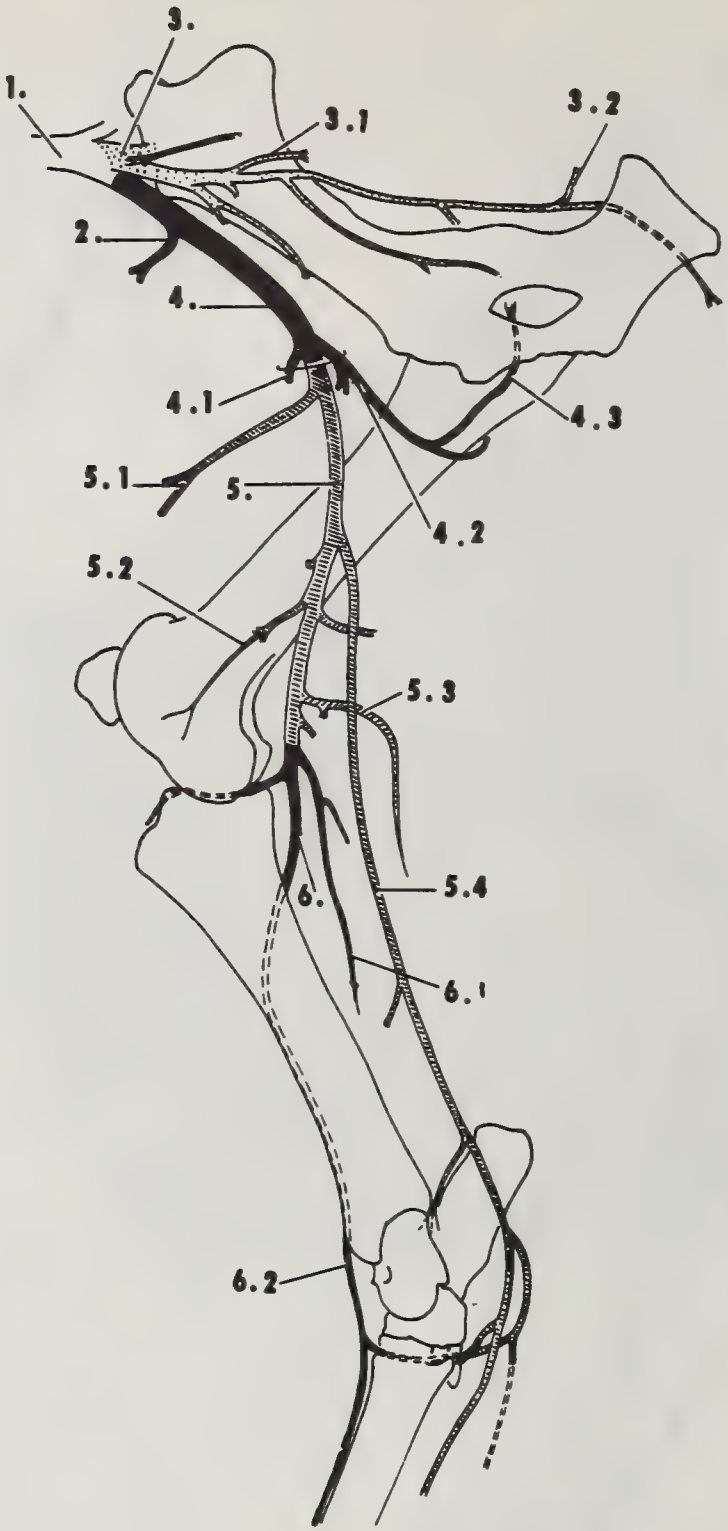


FIGURE 44.—Major arteries of pelvic limb of calf.

DIRECT VISUALIZATION OF THE CORONARY MICROCIRCULATION OF THE LEFT ATRIUM OF THE CAT

R. J. Bing* and K. D. Hellberg**

High-speed cinematography and transillumination were used to investigate red cell velocity of the coronary microcirculation of the left atrial appendage of the cat. The potassium-chloride-arrested, as well as the normally beating heart in vivo, were used. Under both circumstances, countercurrents and asymmetric capillary arrangements were found. This has far-reaching importance for the oxygenation of the heart muscle and makes doubtful previous calculations for the oxygen transport from the capillary to the surrounding tissue. Recruitment, denoting an increase in the number of perfused capillaries, was observed with a rise in perfusion pressure in the arrested heart and following the administration of nitroglycerin and nicotine in the beating heart. Capillary autoregulation was also noticed, since red cell velocity appeared to be independent of perfusion pressure. Definite patterns of red cell velocity in the capillaries of the left atrium of the cat emerged. Nicotine and nitroglycerin had little effect on red cell velocity. However, following hemorrhage alone and after the administration of nitroglycerin following hemorrhage, marked changes in the red cell velocity were seen. Two major peaks in red cell velocity in atrial muscle were observed, the first large one immediately preceding or at the onset of ventricular systole, the second smaller one immediately following ventricular systole. The results illustrate that, using transillumination, it is possible to measure quantitatively red cell velocity in the coronary circulation of the cat under a variety of experimental conditions.

INTRODUCTION***

In the past, studies on the microcirculation have been carried out on a variety of animals and organ systems, such as the frog lung, the tail of the eel, and the stickleback.[1–3] Steven Hales first used the mesentery of the dog, a preparation still frequently employed in the study of the microcirculation.[4] We have chosen the cat's heart from the beginning of our studies.

There are several publications in the literature dealing with the coronary microcirculation, most of them using epiillumination.[5,6] Epiillumination would certainly be the ideal way of visualizing the coronary microcirculation, since it would permit a wide field of observation and would not restrict observations to any particular portion of the heart muscle. From the onset, we were well aware of the advantages of this technique and have attempted for the last year to adapt epiillumination to the heart in order to make it more useful for the study of coronary microcirculation. In the course of these experiments, we have attempted holography, a method of three-dimensional presentation, using laser beams. This method was found to be unsatisfactory because of the appearance of "speckles" peculiar to the coherence light of the laser. We also have tried epiillumination by means of surface illumination with one or several quartz rods or quartz rings. This also was only partially successful because of reflection of light, which made visualization in depth and sufficient resolution and contrast difficult.

Quite recently we have achieved more promising results with another approach to epiillumination, namely the use of light from fiber-optic bundles which conduct light from a xenon arc into a surface layer of a cat's heart with a micro needle. It is too early to predict whether this technique will give adequate resolution and permit enough light for high-speed cinematography.

Why was this particular preparation selected? In the first place, the left atrium is the right size and the muscle is of sufficient tensile

*Huntington Institute of Applied Medical Research, Pasadena, California.

**University of Southern California, Los Angeles, California.

***Supported by grants from the Margaret W. & Herbert Hoover, Jr. Foundation and the American Medical Association Education and Research Foundation.

strength to permit the introduction of the light-conducting system. Secondly, it is thin enough for transillumination and visualization of several muscle layers.

The technique which we employed has been described in numerous publications.[7-9] To summarize briefly, cats anesthetized with sodium pentobarbital were used. Direct observation of the coronary microcirculation was accomplished through transillumination of the left atrial muscle. A hollow glass tube was introduced into the left atrial appendage. This glass tube had a flat upper surface which could be gently directed against the atrial muscle (Figure 1). Through this hollow light pipe, a round quartz rod was introduced. This was slightly smaller than the hollow tube and had a reflecting surface at its intracardiac end which directed the light perpendicular to the axis of the rod and through the atrial muscle toward the objective of the microscope. This resulted in transillumination of a specific area of the left atrial muscle. The light source was a pulsating xenon arc. We made use of an intravital microscope developed by Wayland.[9] This permitted the entire preparation to be moved as a unit with respect to the optical train. In our more recent experiments, microcinematographs were taken on 16 mm color film at a speed of 400 frames per second with a Milliken camera. The linear optical magnification on the film was X30.

The difficulties inherent in visualizing an object which moves in and out of focus are obvious. Efforts are therefore under way to design a servomechanism which will permit the automatic synchronization of the movement of the microscopic objective with that of the heart muscle. This is a joint project of the Jet Propulsion Laboratory, the California Institute of Technology, and our institute.[10] However, even though we have to deal with a continuously moving objective, we obtain several clear frames during both phases of the cardiac cycle by focusing on different layers.

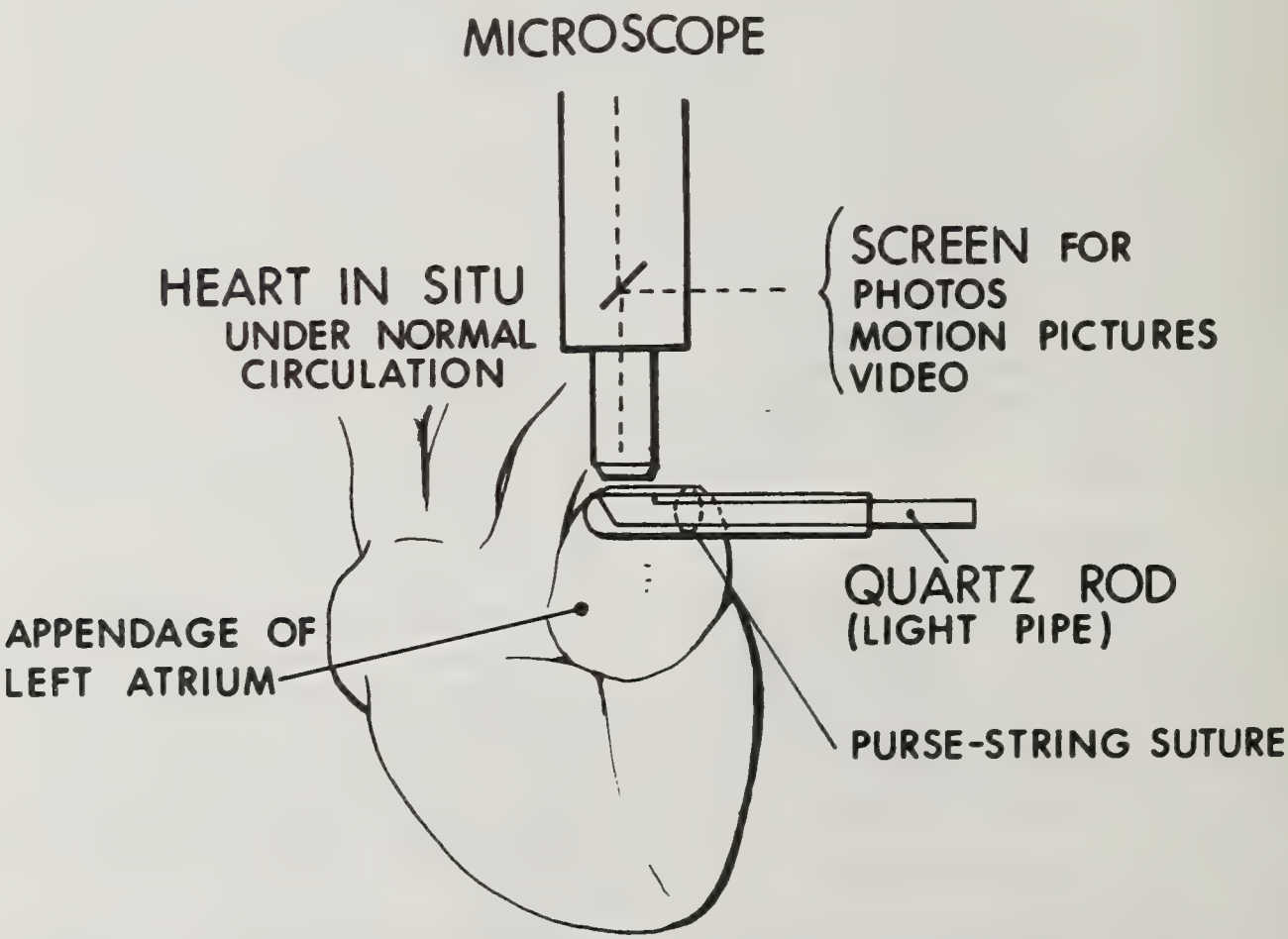


FIGURE 1.

We have repeatedly stressed the difficulties inherent in the method aside from those introduced by a continuously moving objective. There is the uncertainty that the microvascular pattern in the left atrium of the cat, which is supplied by branches of the left circumflex coronary artery, is identical to that in other portions of the heart muscle. A further difficulty is that it is necessary to apply a certain degree of pressure against the muscle in order to prevent flooding of blood between the hollow glass tube and the inner surface of the atrial wall. However, in a series of experiments, we determined that the tension was not sufficient to prevent free movement of red cells in the capillaries. A third criticism which has been raised is that there may be heat conduction from the xenon arc to the wall of the left atrium. We have shown, however, that the temperature at the atrial end of the conducting system differs by less than 2° C from that of the blood. Red cell velocity was measured by frame-to-frame analysis of the movements of red cells by means of a film analyzer. We calculated average data of from between 6 to 40 observations per experimental series. Determinations could only be made on capillaries which remained in focus throughout the cardiac cycle (usually from 3 to 6 capillaries). The data were then averaged and statistically treated.

In a series of experiments, we have observed the microcirculation of the potassium-chloride-arrested left atrium of the cat.[8] Perfusion was carried out by means of a Sigma pump using heparinized cat's blood.

RESULTS AND DISCUSSION

The pattern of the coronary microcirculation is illustrated in Figure 2. Our main efforts were directed toward an investigation of the relationship of flow from one capillary to the other. In 1919, Krogh, in collaboration with Erlang, derived a mathematical solution for steady-state radial diffusion from a cylindrical capillary to the tissue cylinder around it.[11] The Krogh-Erlang equation originated from the assumption that the capillary is infinitely long and straight. It is still used as a fair estimate for an approximate description of the oxygen supply from the capillaries into the surrounding tissues. It therefore assumes a cylindrical symmetry of capillary arrangements with parallel unidirectional flow of blood in the capillaries. On the basis of our studies, these assumptions do not appear to be valid. This is because of the presence of countercurrent flow in the capillaries of the heart and because of asymmetric capillary arrangement.

In the perfused heart, as well as in the normally beating organ in situ, there occurs countercurrent flow in adjacent capillaries. This is the case primarily in capillaries with interconnecting loops. Similar findings have been observed by Grote and Huhmann[12] and by Huhmann and Niesel[13] in the perfused rat heart. In addition, we found that capillaries in overlaying muscle layers form adjoining sheets. Therefore, one can speak of asymmetric capillary arrangement with occasional countercurrents. This arrangement provides a favorable oxygen distribution to the cardiac muscle.

A further observation which is of significance



FIGURE 2.

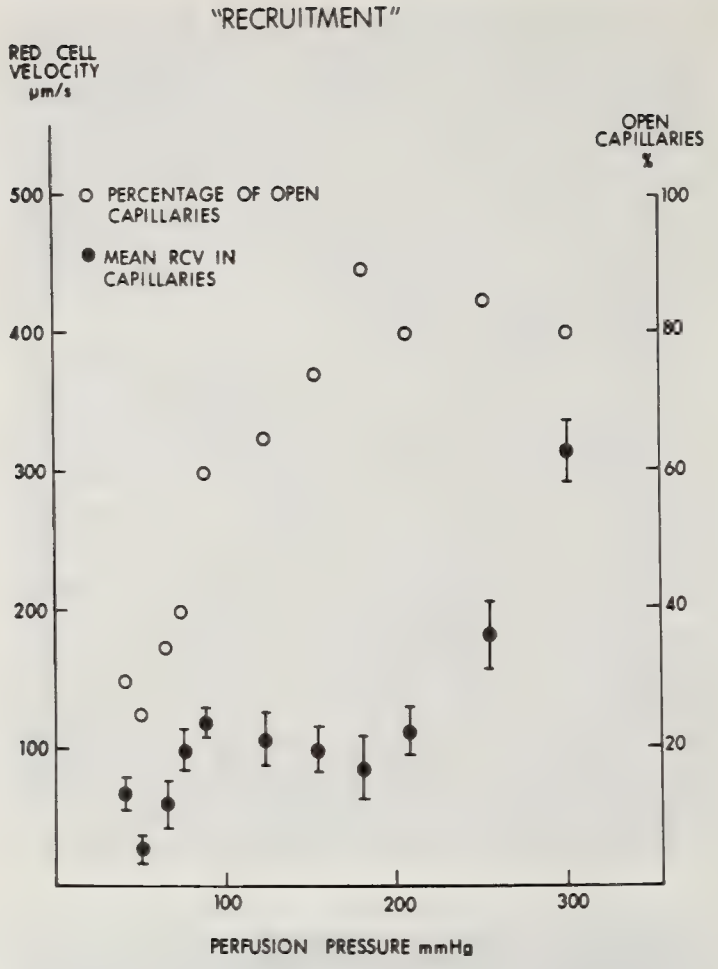


FIGURE 3.

for the oxygenation of heart muscle is "recruitment of capillaries." When the perfusion pressure is raised in the perfused arrested heart, the number of capillaries with discernible red cell movement increases (Figure 3). At the same time, the red cell velocity in individual capillaries remains constant. Similar observations were also made in the beating heart in situ.

Apparently recruitment and autoregulation can occur simultaneously. Autoregulation is here defined as the intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure. It has been observed in the coronary circulation by Cross and Olsson.[14,15] Many investigators have recognized that autoregulation occurs only over a limited range of pressures. Frequently we have observed that when perfusion pressure increases, red cell velocity in individual capillaries remains constant; but because of recruitment, the total oxygen supply to a unit of heart muscle increases.

The cause of recruitment is as yet unknown. It may be a purely mechanical phenomenon. It is more likely, however, that recruitment as well as autoregulation are functions of the metarteriolar muscles and the precapillary sphincters. Direct observations to substantiate this are, however, lacking.

Since our observations were made on the capillary circulation of the left atrium only, no conclusions can be drawn on phasic flow in the coronary microcirculation of ventricular muscle. In addition, red cell velocity varies somewhat between individual capillaries; and, because of movements of the heart, a number of frames may be out of focus, making determinations of phasic flow difficult.

However, a definite pattern for red cell velocity in the capillaries of the left atrium of the cat has emerged (Figure 4). Usually two major

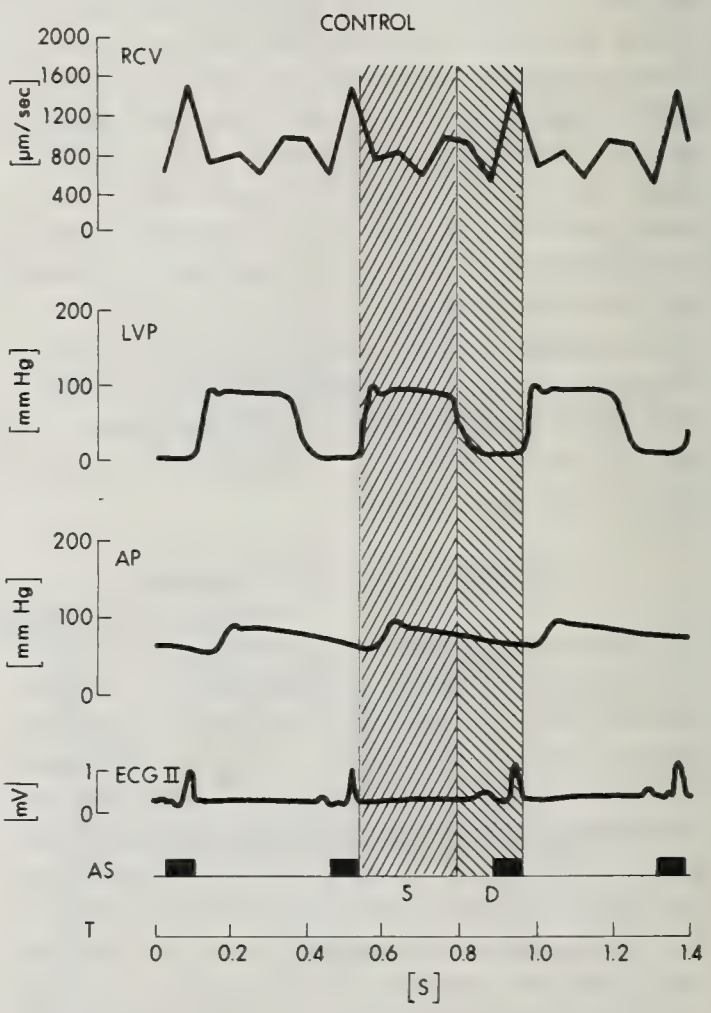


FIGURE 4.

peaks in red cell velocity are seen:[9] the first one immediately precedes or takes place at the onset of ventricular systole; the second is smaller and immediately follows ventricular systole. Therefore the first peak occurs at the end of atrial systole, the second during atrial diastole. Hemorrhage shifts those peaks, with the result that the first occurs at the height of ventricular systole, the second during ventricular diastole. With nicotine, the control pattern is little altered, with the two peaks occurring at the onset and at the terminaion of ventricular systole.

SUMMARY

By means of transillumination, we have been able to study certain features of the coronary microcirculation of the left atrium of the cat. We are aware of the limitations of the method, but we believe that transillumination at the present time is still the best means of obtaining clear pictures of the coronary microcirculation. Efforts are continuing to develop procedures for the visualization of the coronary microcirculation by means of special methods.*

The significant observation of the study is the presence of asymmetric capillary arrangement with countercurrents. This has considerable importance for the oxygenation of the heart muscle. Recruitment and autoregulation may exist simultaneously in the same preparation.

A definite pattern for red cell velocity in the capillaries of the left atrium was observed. Two major peaks in red cell velocity are seen: the first large one immediately precedes or takes place at the onset of ventricular systole; the second smaller one follows ventricular systole. Slight changes in phasic red cell velocity were seen after hemorrhage, nitroglycerin, and nicotine.

REFERENCES

1. YOUNG, J. Malpighi's "De Pulmonibus." Proc. Roy. Soc. Med. 23:1, 1929.
2. VAN LEEUWENHOEK, A. Den Waaragtigen Omloop des Bloeds, als mede dat de Arterien en Venae gecontinueerde Bloed-Vaten zijn, klaar voor de Oogen gestelt Verhandelt in een Brief, geschreven aan de Koninglijke. Societeit tot London, 65th Missive, September 7, 1688. Opuscula selecta Neerlandicorum de Arte medica. Fasc. 1:45, 1907.
3. HALL, M. *A critical and experimental essay on the circulation of the blood; especially as observed in the minute and capillary vessels of the batrachia and of fishes.* London, Seeley and Burnside, 1831.
4. HALES, S. Statical essays: Vol. II. *Containing haemastaticks; or, an account of some hydraulick and hydrostatical experiments made on the blood and blood vessels of animals.* London, W. Innys and R. Manby, 1733.
5. MARTINI, J. and HONIG, C. R. Direct measurement of intercapillary distance in beating rat heart in situ under various conditions of O_2 supply. Microvascular Research, 1:244–256, 1969.
6. HONIG, C. R. Control of smooth muscle actomyosin by phosphate-5 AMP: possible role in metabolic autoregulation. Microvascular Research 1:133–146, 1968.
7. TILLICH, G., MENDOZA, L., WAYLAND, H., and BING, R. J. Studies on the coronary microcirculation of the cat. Amer. J. Cardiol. 27:93, 1971.
8. HELLBERG, K., RICKART, A., WAYLAND, H., and BING, R. J. Coronary microcirculation in the potassium chloride arrested heart. J. Molecular and Cellular Cardiol. 2:221, 1971.
9. HELLBERG, K., WAYLAND, H., RICKART, A. L., and BING, R. J. Studies of the coronary microcirculation by direct visualization. Amer. J. Cardiol. 29: 593–597, 1972.
10. MANSOUR, M., and GREEN, R. Project on the construction of servomechanisms for high-speed cardiac cinematography. In preparation.
11. KROGH, A. Reminiscences of work on capillary circulation. Isis 41:14, 1950.
12. GROTE, J., HUHMANN, W., and NIESEL, W. Untersuchungen ueber die Bedingungen fuer die Sauerstoffversorgung des Myokards an perfundierten Rattenherzen. II. Zur Funktion des Myoglobins. Pfluegers Arch. Ges. Physiol. 294:256–264, 1967.
13. HUHMANN, W. and NIESEL, W. Untersuchungen ueber die Bedingungen fuer die Sauerstoffversorgung des Myokards an perfundierten Rattenherzen. I. Zur Frage nach dem Vorliegen einer Gegenstromversorgung. Pfluegers Arch. Ges. Physiol. 294:250–255, 1967.
14. CROSS, C. E. Influence of coronary arterial pressure on coronary vasomotor tonus. Circ. Res. 15 (Suppl. 1):1–87, 1–93, 1964.
15. OLSSON, R. A. Kinetics of myocardial reactive hyperemia blood flow in the unanesthetized dog. Circ. Res. 15 (Suppl. 1):1–81, 1–86, 1964.

* We have recently been able to visualize ventricular capillaries. Their arrangements do not appear to differ from those observed from the atrial capillary.

CARDIAC EFFECTS OF HEMORRHAGIC SHOCK IN DOGS AND OTHER ANIMALS

D. B. Hackel, J. Chang, N. B. Ratliff and E. Mikat*

The dog has been used extensively in studies of the effects of hemorrhagic shock. The present report will describe the changes seen in the myocardium of dogs and other animals subjected to oligemic hypotension. These include two types of lesions: 1) subendocardial hemorrhage and necrosis, which are predominantly the result of hypoxia; and 2) "zonal" lesions, which apparently result from hemodynamic factors inherent in the shock state. The formation of both lesions is augmented by increased sympathetic activity and is decreased or prevented by blocking catecholamine effects. Zonal lesions are found in cats and pigs subjected to shock, and in a lesser severity in rabbits and humans, but not in rhesus or squirrel monkeys. Subendocardial hemorrhage and necrosis occur in all animals studied except rabbits.

INTRODUCTION**

Studies of the pathological effects of experimental hemorrhagic shock have concentrated on the dog, but numerous descriptions also exist of observations in various animals[1] such as cats, rats, rabbits, monkeys, and goats, as well as in man.[2] It is well recognized that shock involves most organs of the body in all animals that have been studied and, at the same time, it is known that the intensity of involvement of some organs varies between different species. For example, the splanchnic congestion seen prominently in dogs[3] is only a minor feature of the shock syndrome in primates.[4,5] Thus, it is of potential importance to know the comparative pathological effects of shock in different animal species.

DISCUSSION

There has been evidence from numerous physiologic studies of the important contribution of cardiac failure to "irreversibility" of experimental shock.[6] We have studied the character of the anatomic changes that are induced in the hearts of animals subjected to hemorrhagic shock, and have demonstrated two types of lesions that are different in appearance and in etiology: 1) subendocardial hemorrhage and necrosis, and 2) "zonal" lesions.

Subendocardial hemorrhage and necrosis of the myocardium are commonly seen in shock, but are not specific for the shock syndrome. They have been frequently recognized as occurring secondary to situations in which the common elements of increased sympathetic activity and hypoxia are present.[7] We have demonstrated that these lesions can be prevented by blocking beta sympathetic activity,[8] by preventing the usual tachycardia that occurs in shock[9] or by treating the animals with an increased concentration of oxygen at hyperbaric levels.[10]

The second type of myocardial damage that occurs in shocked animals has been referred to as "zonal" lesions.[11–13] They seem to occur only in a setting of oligemic hypotension, and thus are rather specific for shock. The lesions can be seen in routine light microscopic sections, but are obscure and difficult to differentiate from artifacts and contraction bands. However, frozen sections stained for oxidative enzyme activity (such as succinic dehydrogenase) reveal their unique characteristics.[11] They are recognized in the papillary muscles of both ventricles, and in the subendocardial regions mainly of the left ventricle as slightly swollen ends of myocytes that have a clear zone adjacent to the intercalated discs, with a margin of increased enzyme activity. See Figure 1. This appearance is confirmed by electron microscopic study as representing a focal hypercontraction of the ends of some myocytes, with scalloping of the sarcolemma, bizarre bending

*Department of Pathology, Duke University Medical Center, Durham, North Carolina.

**Supported by U.S.P.H.S. grant HL–05875 and carried out during the tenure of a Career Research Award from the USPHS (HL–K6–14,188).

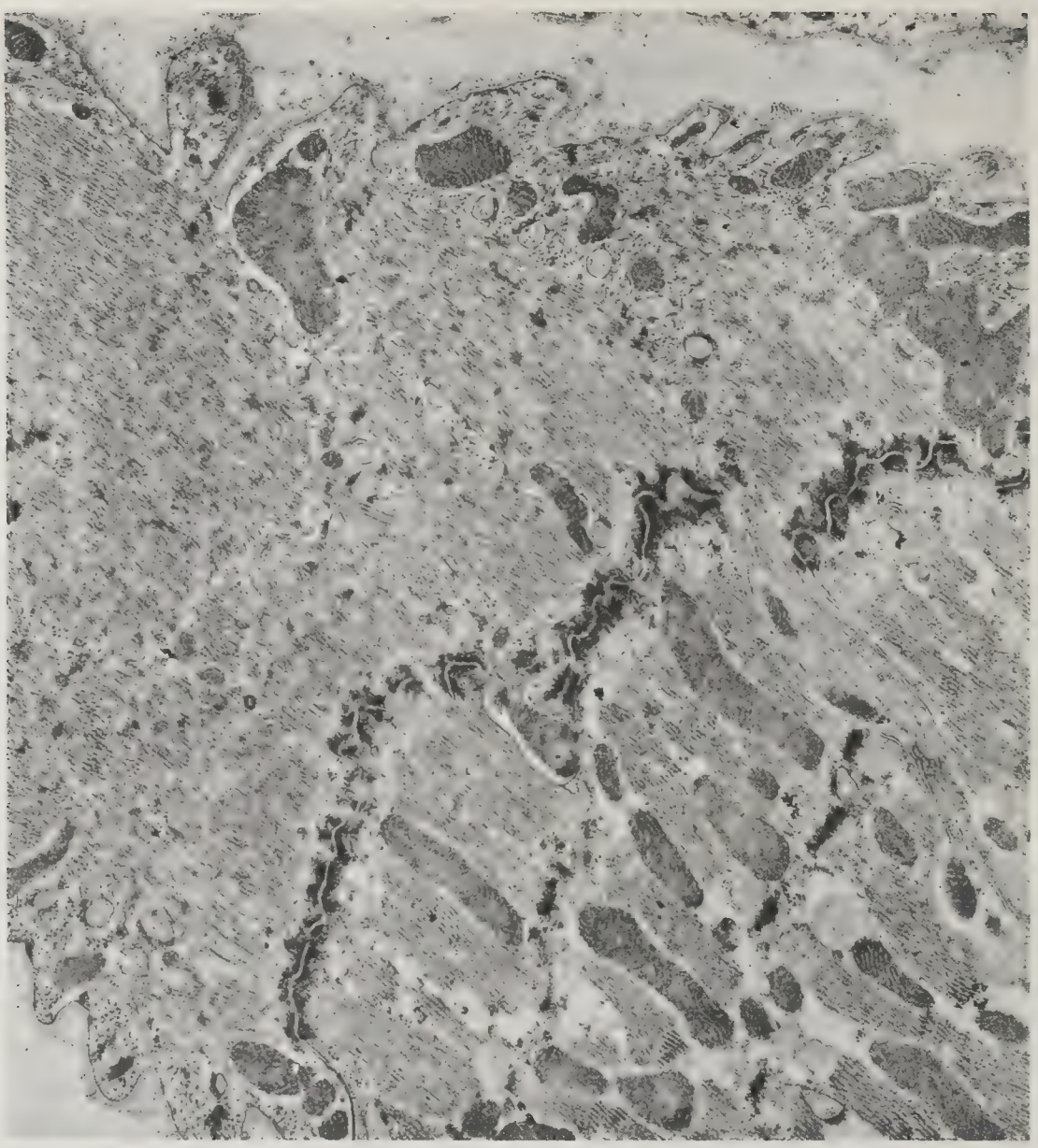


FIGURE 1.—Electron micrograph of myocardium of cat subjected to hemorrhagic hypotension for 1 hour. Note relatively normal myofibers to right and below intercalated disc. The zonal lesion is to the left and above intercalated disc, with fragmented Z bands and translocation of mitochondria which have mostly been displaced to left (out of field of picture). × 14,000.

of myofilaments, disruption of z-band material, and translocation of mitochondria.[12,13] The latter feature accounts for the clear zone seen in the succinic dehydrogenase preparations.

Zonal lesions are seen early in shock. They have been shown to be reversible in their initial stages of development,[13] but they become progressively more severe and extensive when the shock period is prolonged. It is our contention that they represent sufficient damage to the anatomic integrity of the heart to account, at least in part, for the occurrence of eventual cardiac failure and "irreversibility" of the animal to transfusion.

Zonal lesions can be prevented by some of the same manipulations that prevent subendocardial hemorrhage and necrosis. This includes the avoidance of tachycardia by surgically produced heart block,[9] and blockade of beta-sympathetic activity by pronethalol treatment.[8] On the other hand, a clear-cut etiologic distinction is provided by studies in which animals were treated with hyperbaric oxygen. At oxygen concentrations sufficient to significantly augment

TABLE 1.—*Summary of Lesions of Hearts of Animals in Hemorrhagic Shock*

Animal	Zonal lesions*	SEH*
Rabbits	1/6	0/6
Cats	4/5	5/5
Pigs	5/5	5/5
Squirrel monkeys	0/3	1/3
Rhesus monkeys	0/3	2/3

SEH = Subendocardial hemorrhage.
*Ratio of number of animals with the lesion to the number of animals examined.

tissue oxygen supply, there was no decrease in the severity of zonal lesions, although subendocardial hemorrhage and necrosis were almost completely eliminated.[10]

Most of the above studies were carried out in dogs. We have recently, however, made observations on the myocardial lesions seen in several other species subjected to a hemorrhagic shock regime[14] (Table 1). Zonal lesions were seen in rabbits, cats, and pigs; and they have previously been recorded in man.[15] However, they were not seen in either squirrel monkeys or rhesus monkeys. Subendocardial hemorrhage was not seen in 6 out of 6 rabbits subjected to hemorrhagic shock, but was present in all cats and pigs, and in 3 out of 6 monkeys following hemorrhagic shock. The reasons for these apparent species differences are not known, but it would be of interest to seek an explanation by comparing the hemodynamic changes in shock in the different groups.

SUMMARY

The effects of experimental hemorrhagic shock on the hearts of dogs, cats, pigs, rabbits, rhesus monkey and squirrel monkeys were studied. Subendocardial hemorrhage and necrosis are seen in all animals studied except rabbits. "Zonal" lesions are observed in dogs, cats, pigs, and rabbits, but not in rhesus or squirrel monkeys.

REFERENCES

1. ZWEIFACH, B. W.: Annotated bibliography on shock. Publics. 1182 and 1358 of the National Academy of Sciences, National Research Council, Washington, D. C., 1963 and 1966.
2. HARDAWAY, R. M.: Clinical management of shock. Ch. C. Thomas, publ., Springfield, Ill., 1968.
3. HACKEL, D. B., and CATCHPOLE, B. N.: Pathologic and electrocardiographic effects of hemorrhagic shock in dogs treated with l-norepinephrine. Lab. Invest., 7:358–368, 1958.
4. HINSHAW, L. B.: Comparative effects of endotoxin on canine and primate intestine. J. Surg. Res., 8:535–538, 1968.
5. ABEL, F. L., WALDHAUSEN, J. A., and SELKURT, E. E.: Splanchnic blood flow in the monkey during hemorrhagic shock. Am. J. Physiol., 208:265–269, 1965.
6. CROWELL, J. W., and GUYTON, A. C.: Further evidence favoring a cardiac mechanism in irreversible hemorrhagic shock. Am. J. Physiol., 203:248–252, 1962.
7. HACKEL, D. B., and GOODALE, W. T.: Effects of hemorrhagic shock on the heart and circulation of intact dogs. Circ., 11:628–634, 1955.
8. ENTMAN, M. L., HACKEL, D. B., MARTIN, A. M., MIKAT, E., and CHANG, J.: Prevention of myocardial lesions during hemorrhagic shock in dogs by pronethalol. Arch. Path., 83:392–395, 1967.
9. HACKEL, D. B., MARTIN, A. M., SPACH, M. S., and SIEKER, H. D.: Hemorrhagic shock in dogs. Relation of hemodynamic and metabolic changes to myocardial lesions. Arch. Path., 77:575–581, 1964.
10. RATLIFF, N. B., HACKEL, D. B., and MIKAT, E.: The effect of hyperbaric oxygen on the myocardial lesions of hemorrhagic shock in dogs. Am. J. Path., 51:341–349, 1967.
11. MARTIN, A. M., and HACKEL, D. B.: The myocardium of the dog in hemorrhagic shock. Lab. Invest., 12:77–91, 1963.
12. MARTIN, A. M., HACKEL, D. B., and KURTZ, S. M.: The ultrastructure of zonal lesions of the myocardium in hemorrhagic shock. Am. J. Path., 44:127–140, 1964.
13. MARTIN, A. M., and HACKEL, D. B.: An electron microscopic study of the progression of myocardial lesions in the dog after hemorrhagic shock. Lab. Invest., 15:243–260, 1966.
14. CHANG, J., and HACKEL, D. B.: Comparative study of the myocardial lesions in hemorrhagic shock. In preparation.
15. MARTIN, A. M., GREEN, W. B., SIMMONS, R. L., and SOLOWAY, H. B.: Human myocardial zonal lesions. Arch. Path., 87:339–342, 1969.

SUPPLEMENTARY REFERENCES ON EFFECTS OF SHOCK ON THE HEART

16. GOMEZ, O. A., and HAMILTON, W. F.: Functional cardiac deterioration during development of hemorrhagic circulatory deficiency. Circ. Research, 14: 327–336, 1964.
17. ROTHE, C. F., and SELKURT, E. W.: Cardiac and peripheral failure in hemorrhagic shock in the dog. Am. J. Physiol., 207:203–214, 1964.

18. Lefer, A. M., Cowgill, R., Marshall, F. F., Hall, L. M., and Brank, E. D.: Characterization of a myocardial depressant factor present in hemorrhagic shock. Am. J. Physiol., 213:492–498, 1967.
19. Hiott, D. W.: Ultrastructural changes in heart muscle after hemorrhagic shock and isoproterenol infusions. Arch. Int. Pharmacodyn., 180:206–216, 1969.
20. Ratliff, N. B., Hackel, D. B., and Mikat, E.: Myocardial oxygen metabolism and myocardial blood flow in dogs in hemorrhagic shock. Effects of hyperbaric oxygen. Circ. Research, 24:901–909, 1969.
21. Ratliff, N. B., Hackel, D. B., and Mikat, E.: Myocardial carbohydrate metabolism and lesions in hemorrhagic shock: effects of hyperbaric oxygen. Arch. Path., 88:470, 1969.
22. Siegel, H. W., and Downing, S. E.: Reduction of left ventricular contractility during acute hemorrhagic shock. Am. J. Physiol., 218:772–779, 1970.
23. Siegel, H. W., and Downing, S. E.: Contributions of coronary perfusion pressure, metabolic acidosis and adrenergic factors to the reduction of myocardial contractility during hemorrhagic shock in the cat. Circ. Research, 27:875–889, 1970.
24. Hackel, D. B., Martin, A. M., Capp, M. P., Spach, M. J., and Mikat, E.: Effect of pronethalol on left ventricular volumes of dogs during hemorrhagic shock. Am. J. Path., 60:93–101, 1970.
25. Bethea, H. L., Jones, C. E., and Crowell, J. W.: Effect of pharmacologic coronary flow augmentation on cardiac function in hypotension. Am. J. Physiol., 222:95–100, 1972.

DISCUSSION

Chairman Kusserow: Thank you, Dr. Hackel, for your very fine presentation of some of the ultramicroscopic aspects of the shocked state.

Richard E. Clark, Washington University, St. Louis, Mo.: To continue with the subject of anoxia, some years ago Dammond and I published a paper covering a spectrum of animals including rats, guinea pigs, rabbits, dogs, and humans. The earliest sign of subendocardial hemorrhage occurred within the bundle of His which probably has the richest blood supply per square millimeter of any place in the heart. If you kill a rat within two or three minutes by totally flooding a closed environment with nitrogen, for example, then immediately autopsy him and section the bundle of His area, you'll find gross hemorrhage. We found from 30 to 70% in every species studied. We entered this area due to a patient who, when his PO_2 was raised, would go into sinus rhythm. Then when we decreased his PO_2 on a ventilator, he went into an AV block. This was repeated time and time again. I honestly believe that these lesions occur very, very rapidly even though these animals obviously went into a shocked state in a few minutes from the time of initiation of anoxia to death. I also believe hypoxia does play an important role in subendocardial hemorrhage.

Dr. Hackel: Yes, I agree absolutely. I think it does. And I think that it can be caused by several things, such as putting animals in nitrogen. Stellate ganglion stimulation alone will also do it, presumably by its hypoxic effect on the myocardium. So I'd agree that this is true.

PATHOLOGICAL TECHNIQUES APPLIED TO ARTIFICIAL ORGAN RESEARCH. AN ANIMAL MODEL FOR THE DETECTION OF SURFACE-INDUCED THROMBOSIS AND EMBOLISM

B. K. Kusserow*

Selected pathological techniques have been utilized by a number of investigators to study prosthesis-induced thromboembolic phenomena—one of the foremost problems associated with the use of prosthetic circulatory devices.

Prosthesis-induced thrombosis has been investigated in a number of laboratories with a variety of animal test systems. In general the candidate test material or prosthetic device is either implanted into, or coupled directly with the vascular system for varying periods of time. Detailed *ex vivo* observation (optical viewing apparatus) or postmortem examination of the implant or prosthetic device and associated thrombotic material follows.

An animal test model to assess prosthetic devices and synthetic materials for their embolic propensity as well as their tendency to thrombosis is here described. In this preparation the vascular bed and tissues of the kidneys, organs highly susceptible to embolic infarction, are utilized as sensitive biologic indicators of embolic events originating at the surface of an implant. Experience with the test model suggests that thrombosis which occurs on the surface of smooth, rigid intravascular implants may be accompanied by embolic episodes with great frequency, and further that the absence of thrombotic deposit upon the surface of an intravascular implant does not rule out the possibility of antecedent thrombosis with complete embolization of all thrombotic material.

INTRODUCTION

During the past decade considerable effort has been directed to the fabrication of synthetic [1–9] and biologic [10,11] surfaces for use with circulatory assist and vascular prosthetic devices. Progress has been impressive, but a survey of the literature suggests that all such surfaces developed to date possess certain imperfections. The problems of surface-induced thrombosis and subsequent peripheral embolization in particular have stubbornly resisted solution. Prosthesis-induced embolic phenomena represent complications of especial importance in view of the potentially lethal hazards of emboli. This is particularly true within the greater (systemic) circulation where the majority of circulatory assist and intravascular prosthetic devices are positioned. The necessity for thorough evaluation of new surfaces for their thrombogenic and embolic propensities is thus apparent, and suggests the need of a reliable animal testing model for this purpose. It also remains to be added that such evaluation would appear to be of particular importance with respect to either very smooth, rigid surfaces or surfaces which flex constantly. In such cases the possibility exists that thrombotic deposits may be dislodged from the surface with increased frequency, due either to poor mechanical attachment (smooth, rigid surfaces) or fragmentation due to incessant wall movement (flexing surfaces).

A variety of animal test systems have been utilized to date (1) for the study of surface-induced thrombosis and (2) for the evaluation of promising new synthetic and biologic surfaces with respect to their ability to retard thrombosis. The basic approach used in such models involves either implantation into or direct coupling with the host vascular system for varying periods of time. Several investigators have utilized rigid cylindrical rings of the candidate test material for purposes of evaluation. Rings have been implanted either into the vena cava,[12,13] aorta [14,15] or major aortic branch.[16] These vessels have also served as the major test sites for fabric vascular graft materials.[17] New materials have also been evaluated within the cardiac chambers in the form of sword-like projections arising from the atrial wall.[18] Other workers have incorporated new

*Department of Pathology, University of Vermont College of Medicine, Burlington, Vermont.

materials or surfaces into extracorporeal shunts for purposes of evaluation.[19] Promising materials for cardiac valve surfaces have generally been assessed by intracardiac implantation at the specific valve site projected for their use,[20] while newly developed biologic surfaces for prosthetic devices have often been assessed while serving as blood contacting parts and lining surfaces for circulatory assist devices.[21,22] Finally, resort has also been made to the use of *ex vivo* flow chambers and circuits for both the appraisal of synthetic surfaces and the study of early events in the process of thrombosis.[23,24]

It is noteworthy that in all of the systems cited above primary emphasis has been placed upon the amount of thrombotic deposit encountered *in situ* upon the surface of the implant under study. Embolic phenomena generally have not been investigated in detail. Indeed most of the systems referenced above appear to be less than optimally suited for the detection of embolic episodes. This is due to the fact that in these preparations emboli of implant origin are not obligatorily channeled to receptor vascular beds which represent satisfactory biologic indicator systems for the consistent documentation of embolic episodes. Minute emboli are particularly difficult to localize and identify within the peripheral vascular channels of most systemic organs. Lessor embolic episodes are also difficult to document within the pulmonary vascular bed, due first to the dual nature of the pulmonary circulation which protects the lung against embolic infarction,[25] and secondly to the action of the host fibrinolytic system which tends to bring about rapid dissolution of pulmonary emboli.[26]

PATHOPHYSIOLOGICAL BASIS FOR AN IN VIVO EMBOLUS TEST SYSTEM

It has long been recognized that the tissues of various organs differ markedly with respect to their susceptibility to embolic infarction. Those tissues which possess extensive collateral circulation are relatively resistant to infarction, while those tissues which possess an end organ circulatory pattern characterized by little or no collateral circulation have been known to be unusually susceptible to embolic infarction. This circumstance is due to the fact that tissue perfusion largely ceases in a given mass of tissue with little or no collateral blood supply, when an occlusive embolic particle lodges within its definitive artery of supply. Ischemic necrosis or infarction generally follows, and leaves a permanent anatomical marker of the embolic event. The pathophysiological relationships which exist in tissues possessing such an end organ circulatory pattern provide the basis for their use in animals as *in vivo* models for the detection of prosthesis-induced embolic events. Thus, if a given prosthetic surface is positioned in the path of blood which obligatorily courses through an organ possessing an end organ circulatory pattern, emboli arising from the surface of the implant will be entrained within the blood stream, cause subsequent arterial occlusion, cessation of adequate perfusion and the production of tissue infarction. The latter will remain as a permanent marker of the embolic event.

RENAL EMBOLUS TEST SYSTEM

Design, Structure and Operation of the Model

The relationships cited above have been utilized in the development of an *in vivo* model for the detection not only of thrombotic deposit actually present upon the surface of a prosthetic implant, but also of embolic phenomena originating at the implant site. The canine renal vascular bed and tissues were selected as an indicator system for the identification of embolic events, because of the known vulnerability of these organs to embolic infarction. They represent organs which readily display even minute infarcts and can be conveniently examined in great detail by conventional pathological techniques.[27]

The surface or material to be evaluated, prefabricated as a cylindrical ring implant with carefully beveled edges, is inserted into the abdominal aorta immediately above the origin of the renal arteries. The aorta is then subtotally constricted (2–3 mm residual lumen) below the level of the renal arteries to divert

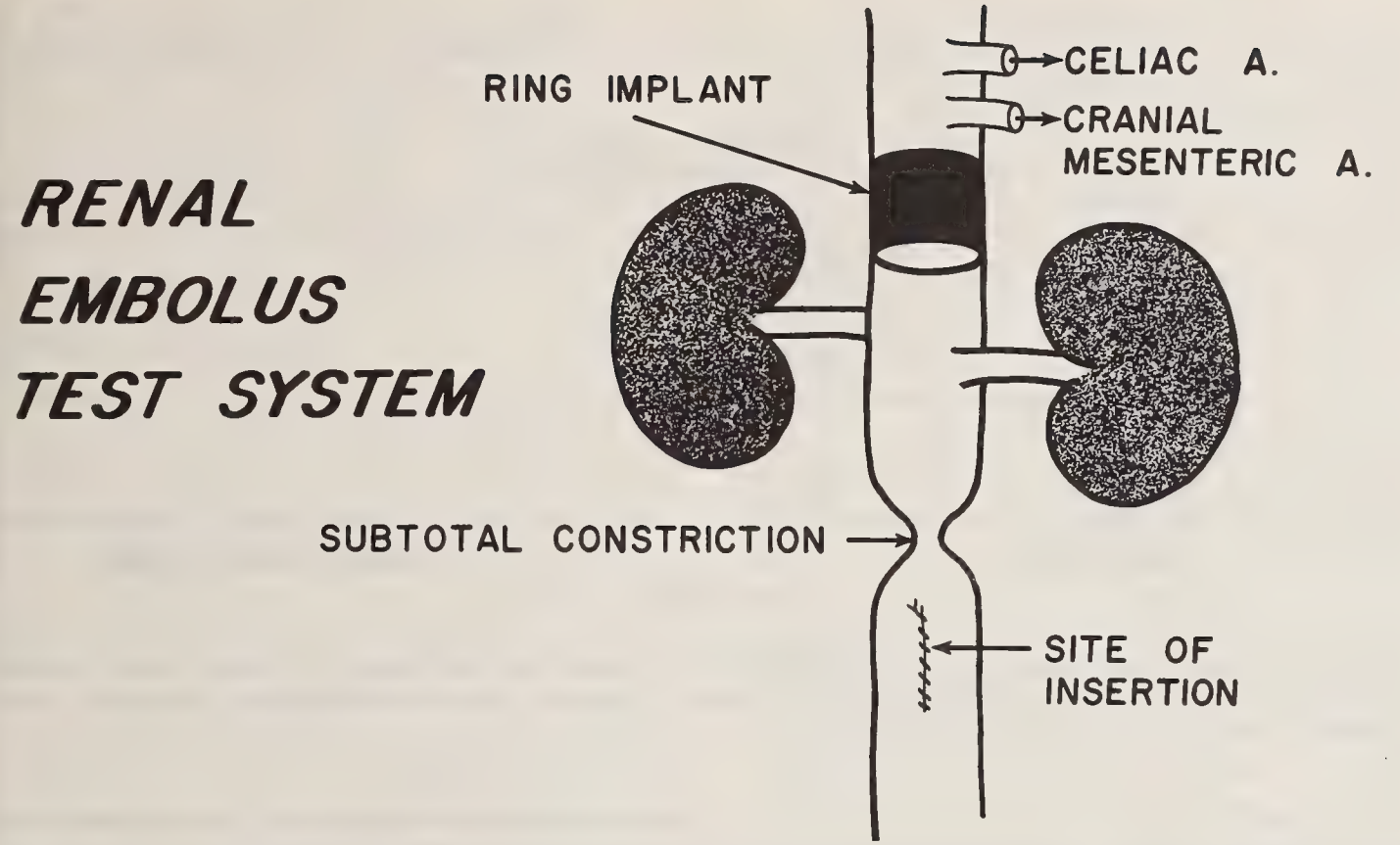


FIGURE 1.—The renal vascular bed and tissues serve as a biologic indicator system to document embolic episodes at the ring implant site. Subtotal infrarenal aortic constriction diverts the large majority of emboli to the kidneys.—From Kusserow, Larrow and Nichols.[27]

most of the aortic flow at this level into the renal vascular bed (Figure 1). The aorta is also carefully wrapped with fabric at the implant site to eliminate any potential dead space between the outer surface of the ring implant and the inner surface of the pulsating aorta which might serve as a site for adventitious thrombosis. More complete details of the implantation procedure have been described in a previous communication.[27] Evaluation of the test ring follows implantation periods of 3–5 days, at which time the animal is systemically anticoagulated with intravenous heparin (5 mg/kg) and promptly sacrificed. Both kidneys, renal arteries and the attached segment of aorta (from above the level of the implant to a level well below the constriction and insertion sites) are removed en bloc. The ring implant and tissues of the test system are then examined both grossly and microscopically for evidence of thrombosis or embolization (renal infarcts).

Hemodynamic Conditions at the Implant Site

Selected pressure and flow measurements were carried out at the implant site to determine the nature of the hemodynamic environment encountered by the surface under evaluation. The influence of subtotal infrarenal aortic constriction (as described above) upon aortic pressure pattern at the implant site is demonstrated in Figure 2. Aortic pressure levels show relatively little change, although moderate blunting of the systolic peaks is apparent following constriction. Flow patterns at the implant site on the other hand appear to be significantly modified following constriction (Figure 3). Peak velocities are significantly reduced, and there is virtual elimination of the back flow component. Measured total renal blood flow appears to be well maintained, however. Flow measurements further show that approximately 90% of blood which passes through the aortic implant is diverted to the kidneys following the aortic constriction maneuver.

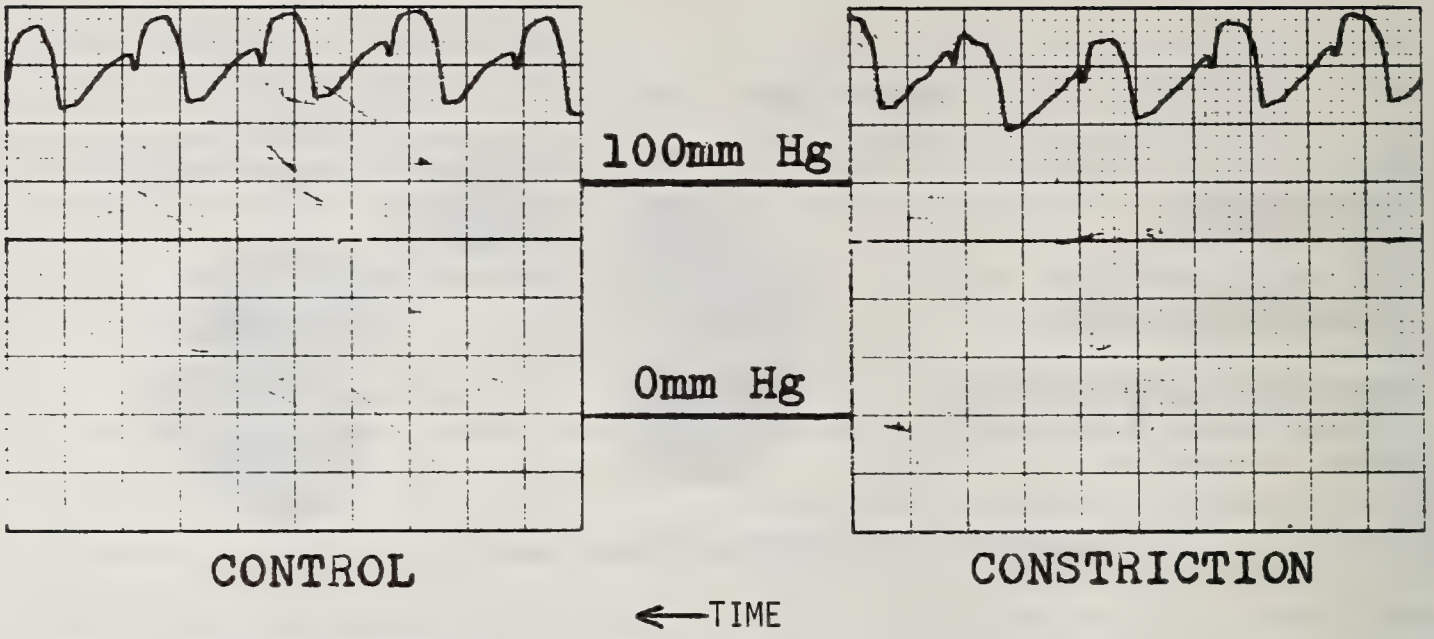


FIGURE 2.—Subtotal aortic constriction (as shown in Figure 1) produces little change in measured aortic pressure levels at the ring implant site, although minor blunting of systolic peaks is evident. From Kusserow, Larrow and Nichols.[27]

Control Experiments

Two series of control experiments were conducted to evaluate the test system with respect to the production of spontaneous or artifactual thrombosis, embolization and renal infarcts (falsely positive results). A third group of control procedures were carried out to determine whether or not the model would respond reliably to embolic episodes by the consistent development of renal infarcts.

The first group of control procedures consisted of subtotal infrarenal aortic ligation without ring insertion in three dogs. No renal infarcts were produced. Nor were renal infarcts observed following a sham insertion procedure (eight dogs) consisting of subtotal

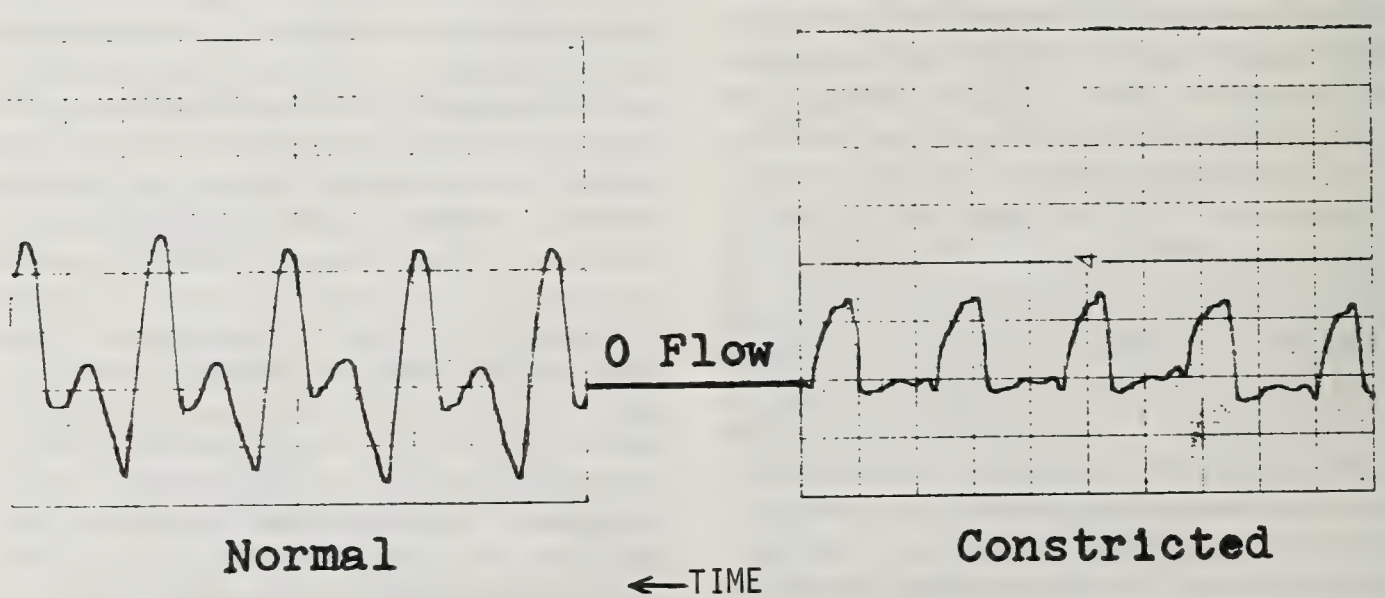


FIGURE 3.—Aortic flow patterns at the implant site are distinctly modified by subtotal infrarenal aortic constriction (as shown in Figure 1). There is reduction of peak flow velocity and nearly complete elimination of the backflow component following the constriction procedure. From Kusserow, Larrow and Nichols.[27]

aortic constriction combined with the brief insertion and withdrawal of a test ring. In contrast numerous renal infarcts were observed in all five animals of a third control series in which subtotal infrarenal aortic ligation was combined with the injection of particulate autologous blood clot into the aorta immediately above the level of the renal arteries.

Use of the Test Model

The renal embolus test system has been utilized in the evaluation of a large number of synthetic surfaces for their thromboembolic propensity. These have included polyethylene, Teflon, polyvinyl chloride, silicone rubber, polyvinyl chloride coated with graphite-benzalkonium chloride-heparin, fluorinated silicone rubber, polyurethane, epoxy, saturated hydrocarbon block copolymer and hydrogel grafted polyurethane. All of these materials were characterized by a relatively smooth, stationary and rigid blood-materials interface. To date all samples of these rigid synthetic materials with smooth surfaces have demonstrated some thrombotic surface deposit, renal infarcts, or a combination of both following implantation periods of 3–5 days in the test model. Ring thrombosis has varied in severity from minor precipitates, encountered frequently along the edges of the ring implants, to nearly complete occlusion of the ring by large thrombotic aggregates which covered major fractions of the luminal surface of the implant. Thrombi were accompanied in well over 90% of instances by unilateral or bilateral renal infarcts. Some representative samples of the thromboembolic patterns encountered are presented in Table I.

Renal infarcts varied considerably in size ranging from less than 1 mm in maximal dimension to nearly complete infarction of a kidney (Figure 4). One ring (GBH coated polyvinyl chloride) was grossly free of thrombus, but the kidneys demonstrated several renal infarcts (Figure 5). In general the thromboembolic patterns observed to date with this test model suggest that thrombosis which occurs on rigid, synthetic intravascular implants with smooth surfaces may be attended by embolic episodes with great frequency. Moreover, the composite findings of ring implants completely free of thrombus in the presence of renal infarcts indicates that the absence of thrombotic material on the surface of intravascular prosthetic devices in no way rules out the possibility of antecedent thrombosis

TABLE I.—*Prosthesis-Induced Thromboembolic Patterns Produced in the Renal Embolus Test System by Various Materials**

Experiment	Ring implant material	Ring thrombosis	Renal infarcts
1	Polyethylene	Lumen 2/3 occluded by thrombus.	Many pale infarcts of varying size in both kidneys.
2	Teflon	Ring lined with thrombus about 2 mm thick. Protrudes caudally.	No infarcts in either kidney.
3	Silicone rubber	Ring lumen nearly occluded by upper and lower rim of ring.	Several infarcts in both kidneys.
4	Silicone rubber	Ring lumen clear. Thrombus on upper and lower rim of ring.	Both kidneys extensively infarcted.
5	GBH coated polyvinyl chloride	Thrombus on distal rim and in lumen of ring.	Scattered infarcts in both kidneys.
6	GBH coated polyvinyl chloride	Ring completely thrombus free.	Numerous infarcts in left kidney. Fewer in right kidney.
7	Fluorosilicone polymer	Ring lumen coated by thin thrombotic deposit which projects caudally from ring.	Several small infarcts in right kidney. None in left kidney.
8	Epoxy	Two to three mm patch of thrombus on caudal rim of ring.	Right kidney almost totally infarcted. Left kidney 24-30% infarcted.
9	Polyurethane coated epoxy	Thin thrombotic deposit on lower rim.	Several infarcts of varying size in both kidneys.
10	Saturated hydrocarbon block copolymer	Minor irregular thin deposit of thrombus on surface of lumen.	Both kidneys free of infarcts.

*From Kusserow, B.[28]

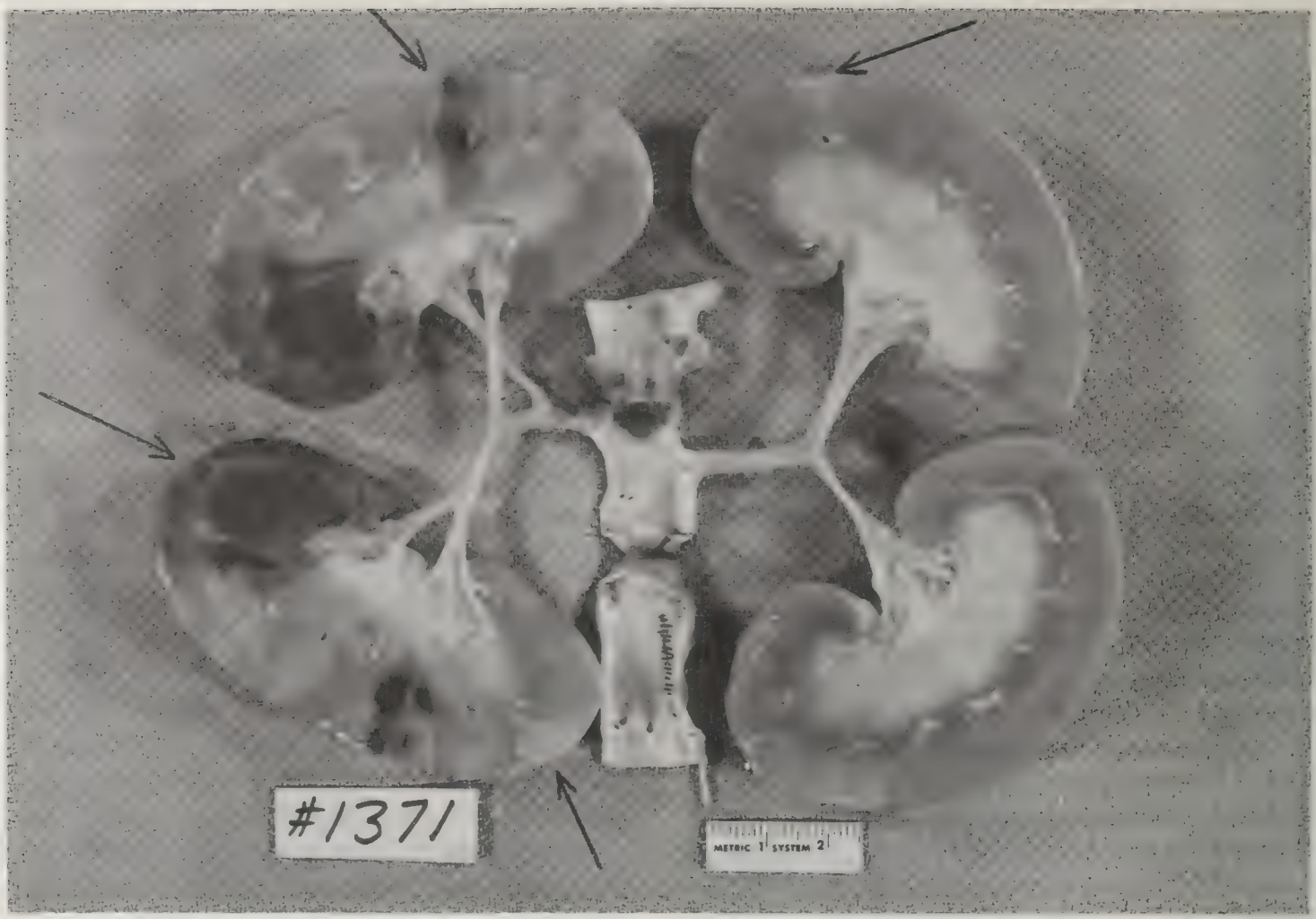


FIGURE 4.—Dissected postmortem specimen of the test model. There are bilateral renal infarcts of varying size, and thrombotic material protrudes from the lower end of the ring implant (seen *in situ*). The aorta has been left uncut at the point of subtotal constriction.

with complete detachment and embolization of all thrombotic material.

DISCUSSION

Experience to date with the renal embolus test system suggests that it may represent a feasible approach for the *in vivo* evaluation of prosthetic surfaces with respect to their thromboembolic propensities. It is worthy of note, however, that the test system is subject to a number of complications which may give rise to misleading results and conclusions, unless appropriate precautionary measures are instituted and the limits of the system are recognized. Thus, scuffing or abrasion of the aortic endothelium near the implant site may initiate small foci of thrombosis on the aortic intimal surface. These may propagate to involve the surface of the ring implant, or fragment to form emboli and subsequent renal infarcts. This complication has been largely avoided by gentle and careful insertion of the test ring. In this regard the implantation procedure has been greatly facilitated by the use of a mounting probe during insertion of the ring implant (Figure 6). Rheologic complications may arise at the edges of an improperly beveled, blunt test ring during the post-operative implantation period. Turbulent flow and eddy currents, caused by the presence of such blunt ring edges, may result in thrombotic deposits which are largely rheologic in origin and not due primarily to the intrinsic nature of the surface under study. To circumvent this difficulty ring implant edges have been carefully beveled. The

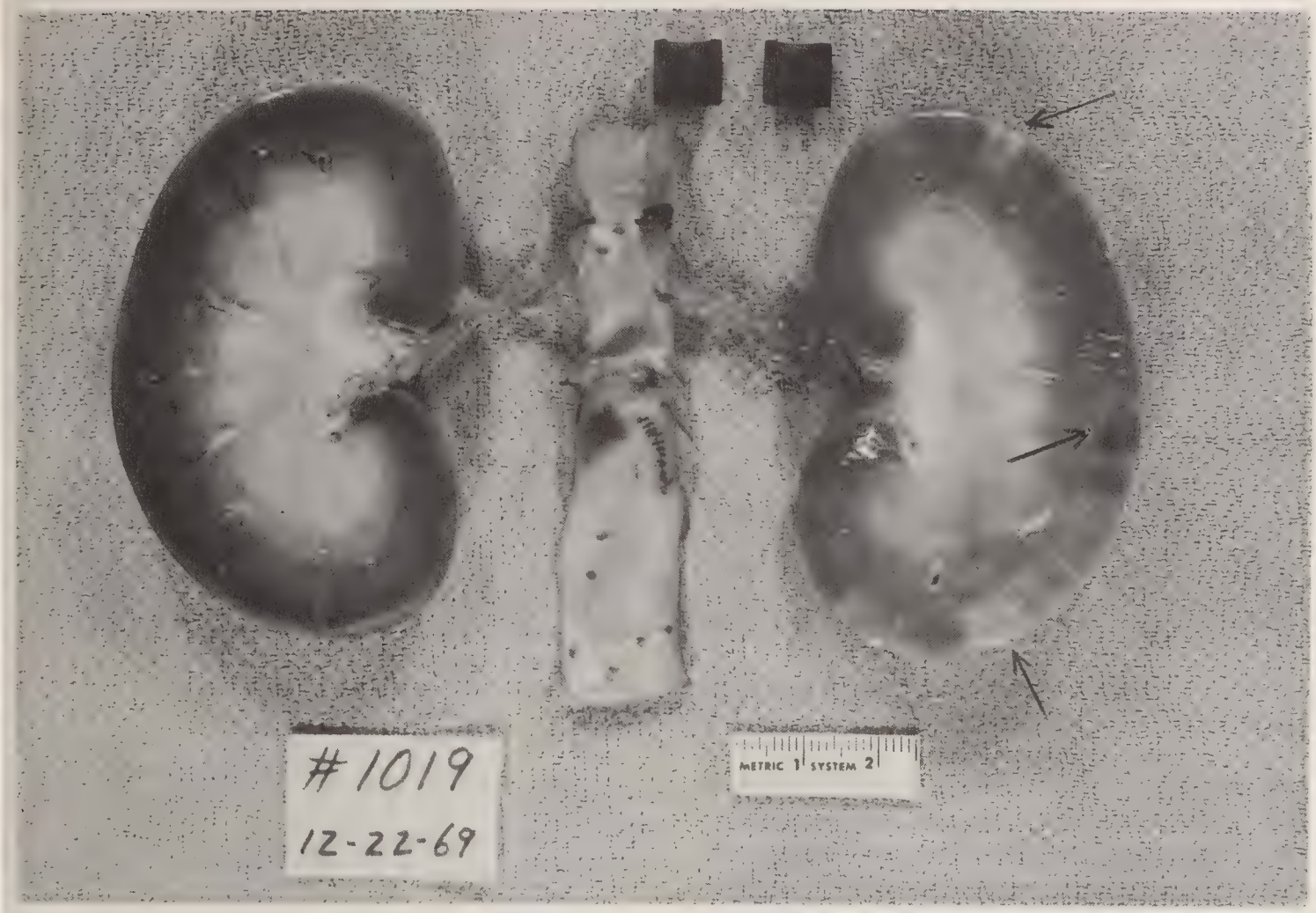


FIGURE 5.—Postimplantation specimen of the test system from an animal with several renal infarcts. The ring implant (shown divided in Figure), however, was grossly free of thrombus. These findings suggest antecedent ring thrombosis with complete detachment and embolization of all thrombus. From Kusserow, Larrow and Nichols.[27]

potential dead space between the outer surface of the ring implant and the adjacent pulsating aortic wall also represents a location for adventitous thrombus formation. Elimination of this dead space has been accomplished by careful wrapping of the aorta with fabric at the implant site.

Difficulties may also arise if the test ring is too large or is malpositioned in such fashion that the ring partially obstructs renal or mesenteric arterial blood flow. This complication can generally be avoided by selection of a test animal of adequate size and careful positioning of the ring implant by means of the mounting probe. It also remains to be commented that a certain measure of experimental variation is unavoidable because of inherent differences in host thrombotic propensity from animal to animal. In a recent study, however, such host factors as whole blood coagulation time, hematocrit, white blood count and platelet count could not be directly correlated with the amount of thrombus produced upon caval and intra-aortic polished quartz ring implants.[12]

The potential complications which have been cited represent compelling reasons for the exercise of appropriate care and caution in the interpretation of pathologic findings encountered with the use of the renal embolus test system. It is also clear that adequate evaluation of a given candidate material or surface is predicated upon the implantation of a sufficiently large number of samples to establish a reasonably reproducible trend or pattern of thrombo-

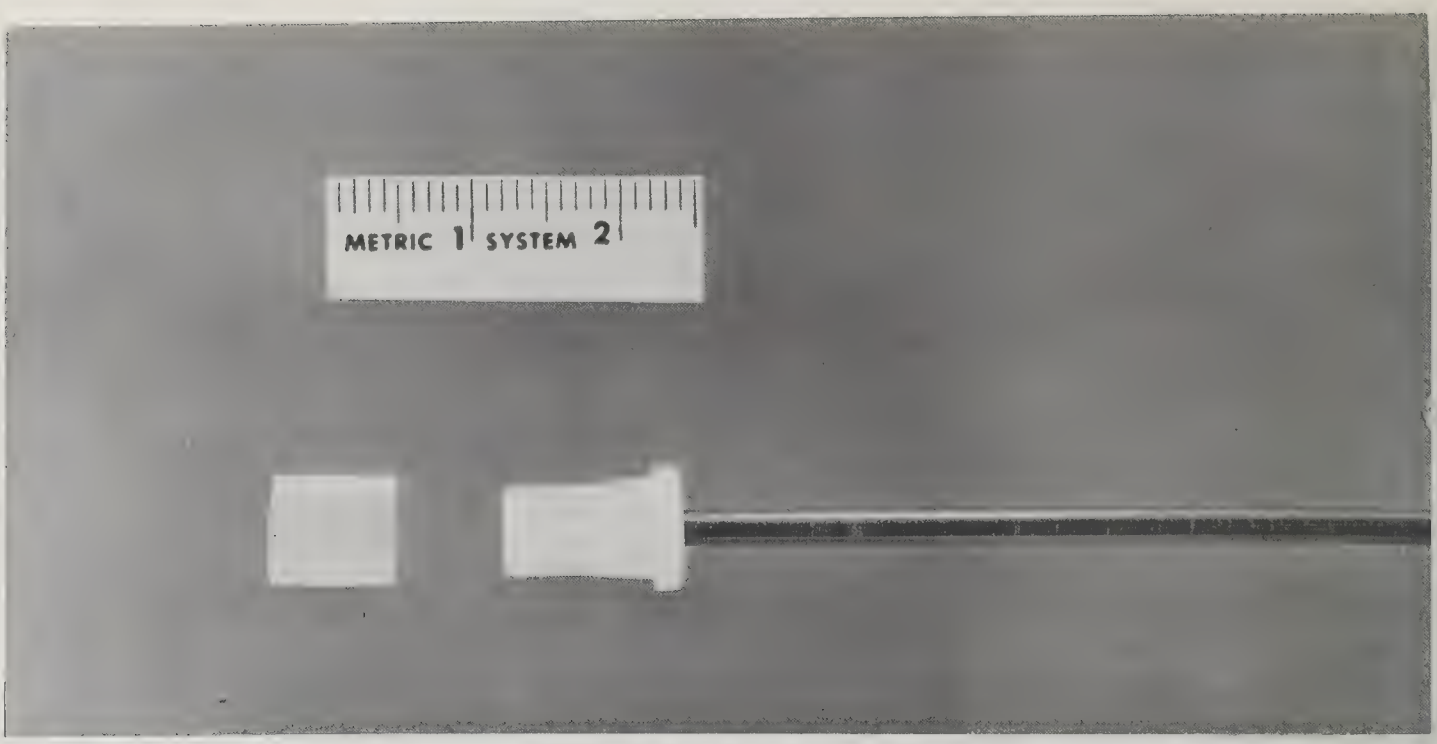


FIGURE 6.—Probe used to stabilize and guide ring during intra-aortic insertion procedure.

embolic phenomena. Despite the restrictions cited above, it seems reasonable to assume that satisfactory performance in an *in vivo* test model of this nature would appear to be a necessary requirement for acceptable resistance to thrombosis and embolus formation.

REFERENCES

1. BIXLER, H., CROSS, R., and MARSHALL, D. Polyeletrolyte complexes as antithrombogenic materials. Proc. Artif. Heart Program Conf. (ed. R. Johnson Hegyeli), 1969, pp. 79–86.
2. SHARP, W., GARDNER, D., and ANDRESEN, G. A bioelectric polyurethane elastomere for intravascular replacement. Trans. Amer. Soc. Artif. Int. Organs 12:179–182, 1966.
3. MURPHY, P., HOLLY, F., VAN SOMEREN, L., BANKOLE, M., SINGH, S., and BERNHARD, W. Antithrombogenic properties of electrified polymers. Trans. Amer. Soc. Artif. Int. Organs 13:131–137, 1967.
4. HALPERN, B., CHENG, H., KUO, S., and GREENBERG, H. Hydrogels as nonthrombogenic surfaces. Proc. Artif. Heart Program Conf. (ed. R. Johnson Hegyeli), 1969, pp. 87–98.
5. MERRILL, E., SALZMAN, E., WONG, P., and ASHFORD, T. Polyvinyl alcohol-heparin hydrogel "G." J. Appl. Physiol. 29:723–730, 1970.
6. GOTT, V., WHIFFEN, J., KOEPKE, D., DAGGETT, R., BOAKE, W., and YOUNG, W. Techniques of applying a graphite-benzalkonium-heparin coating to various plastic surfaces. Trans. Amer. Soc. Artif. Int. Organs 10:213–217, 1964.
7. GRODE, G., ANDERSON, S., GROTTA, H., and FALB, R. Nonthrombogenic materials via a simple coating process. Trans. Amer. Soc. Artif. Int. Organs 15: 1–6, 1969.
8. LYMAN, D., BRASH, J., CHAIKIN, S., KLEIN, K., and CARINI, M. The effect of chemical structure and surface properties of synthetic polymers on the coagulation of blood. II. Protein and platelet interaction with polymer surfaces. Trans. Amer. Soc. Artif. Int. Organs 14:250–255, 1968.
9. KUSSEROW, B., LARROW, R., and NICHOLS, J. The urokinase-heparin bonded synthetic surface. An approach to the creation of a prosthetic surface possessing composite antithrombogenic and thrombolytic properties. Trans. Amer. Soc. Artif. Int. Organs 17:1–5, 1971.
10. BERNHARD, W., HUSAIN, M., GEORGE, J., and CURTIS, G. Fetal fibroblasts as a substratum for pseudoendothelial development on prosthetic surfaces. Surgery 66:284–290, 1969.
11. GHIDONI, J., LIOTTA, D., HALL, C., O'NEAL, R., and DEBAKEY, M. *In vivo* culture of tissue fragments which produce viable cellular linings covered by endothelium (neointimas) in impermeable, velour-lined arterial prostheses, bypass pumps, and valvular prostheses. Trans. Amer. Soc. Artif. Int. Organs 14:69–72, 1968.
12. FOSTER, E., and DOBELL, A. Evaluation of rigid rings as *in vivo* intravascular thrombogenic models. J. Surg. Res. 11:550–558, 1971.

13. Gott, V., Koepke, D., Daggett, R., Zarnstoff, W., and Young, W. The coating of intravascular plastic prostheses with colloidal graphite. Surgery 50:382–389, 1961.
14. Sawyer, P., and Srinivasan, S. Metallic and plastic prosthetic devices as vascular wall substitutes: Biophysical criteria and methods for evaluation. J. Biomed. Mat. Res. 1:83–101, 1967.
15. Sawyer, P., Wu, K., Wesolowski, A., Brattain, W., and Bobby, P. Long term patency of solid-wall vascular prostheses. Surgery 91:735–742, 1965.
16. Sharp, W. The carotid artery—a test site for small vessel prosthetics. J. Surg. Res. 10:41–46, 1970.
17. Wesolowski, A. Evaluation of Tissue and Prosthetic Vascular Grafts. Springfield, Charles C. Thomas, 1962, pp. 77–106.
18. Jacobs, L., Kloop, E., and Gott, V. Studies on the fibrinolytic removal of thrombus from prosthetic swords. Trans. Amer. Soc. Artif. Int. Organs 14: 63–67, 1968.
19. Evans, G., and Mustard, J. Platelet surface reaction and thrombosis. Surgery 64:273–280, 1968.
20. Stuckey, J., Newman, M., Berg, E., Goodman, S., and Dennis, C. Design and placement of prosthetic valves to fit in the mitral ring of the dog after excision of the leaflets. In Merendino, K. (Ed.): Prosthetic Valves for Cardiac Surgery. Springfield, Charles C. Thomas, 1961, pp. 266–276.
21. Bernhard, W., Husain, M., Robinson, T., Button, L., Frieze, S., and Curtis, G. An appraisal of blood trauma and the blood-material interface following prolonged assisted circulation. J. Thoracic and Cardiovas. Surg. 58:801–810, 1969.
22. Ghidoni, J., Liotta, D., Hall, C., O'Neal, R., and DeBakey, M. *In vivo* culture of tissue fragments which produce viable cellular linings covered by endothelium (neointimas) in impermeable velour-lined arterial prostheses, bypass pumps, and valvular prostheses. Trans. Amer. Soc. Artif. Int. Organs 14:69–72, 1968.
23. Lyman, D., Klein, K., Brash, J., and Fritzinger, B. The interaction of platelets with polymer surfaces. I. Uncharged hydrophobic polymer surfaces. Thromb. Diath. Haemorrh. 23:120–128, 1970.
24. Petschek, H., and Adamis, D. Observation of thrombus formation on artificial surface with a controlled flow of unactivated blood. Fed. Proc. 27: 627, 1968.
25. Parker, B., and Smith, J. Pulmonary embolism and infarction. Am. J. Med. 24:402–427, 1958.
26. Allison, P., Dunnhill, M., and Marshall, P. Pulmonary embolism. Thorax 15:273–279, 1960.
27. Kusserow, B., Larrow, R., and Nichols, J. Observations concerning prothesis-induced thromboembolic phenomena made with an *in vivo* embolus test system. Trans. Amer. Soc. Artif. Int. Organs 16:58–62, 1970.
28. Kusserow, B. The use of pathologic techniques in the evaluation of emboli from prosthetic devices. Bul. N.Y. Acad. Med. (In press) 1972.

DISCUSSION

Perry L. Blackshear, University of Minnesota, Minneapolis: Did you have any rings that remained clean, or that left the kidneys clean?

Dr. Kusserow: Yes.

Dr. Blackshear: So this is reasonably good evidence that by squeezing the outside of the aorta enough tissue juice was not produced to incite some sort of rapid clotting phenomena?

Dr. Kusserow: Yes, we think that his so-called "ring end phenomena" to which you refer can be a real problem under two circumstances. One is any sort of traumatic insertion of the ring. This is something of which one must be very careful when the ring is inserted. One can do no better than to use his best surgical judgment and technique and be careful not to injure the endothelial surface. The second thing that can be done to reduce this possibility, is to do away with the trauma which may result from aortic pulsation against the edges of the ring implant. This is the reason that we have used the wrapping. Interestingly enough, Dr. Blackshear, the rings which have remained clear have generally been very smooth. I don't know whether there is anything fundamental behind this or whether the material tends to slide off more easily.

Dr. Blackshear: What materials were those that remained clear without embolizing?

Dr. Kusserow: No material tested to date has yielded both a thrombus free ring and infarct free kidneys during a single implantation procedure.

Jeffrey L. Peters, University of Utah, Salt Lake City: Dr. Kusserow, I enjoyed your paper. I want to ask you two questions. One, have you been able to characterize the different types of thrombi as a function of material? Is this the next stage, or will you be doing this? Secondly, do you have a problem with sepsis with these experiments? Do you have bacteremia sometimes?

Dr. Kusserow: In answer to your first question, we have not been able to find consistent differences which are a function of the type of material, i.e., consistent differences in the composition of the thrombus. They are all rich in platelets. There are also varying amounts of

deposited fibrin, but we have not yet been able to discern differences as a function of type of material. With regard to your second question, all of these procedures are done under aseptic or sterile conditions, so we do not believe that sepsis is a problem.

DR. PETERS: Have you developed a model for the venous system as yet? There are those that might argue that the severity of the test is perhaps not as sensitive or as severe as it should be considering the velocity and the characteristics of arterial blood.

DR. KUSSEROW: There undoubtedly is a difference in the propensity of arterial and venous blood to form thrombi. We would strongly recommend Dr. Gott's system for testing venous thrombosis on surfaces. The problem here is that it is difficult to assess the embolic propensity of implants which are positioned in the venous circulation. This is because one cannot detect them easily in the lungs. Small pulmonary emboli are very difficult to identify.

QUESTIONER (Unidentified): What would you say about the role of the downspeed flow? The second question is what type of sterilization do you use?

DR. KUSSEROW: If I understand your first question correctly, you would like to know whether altered rheology at the implant site might account for some of the embolic phenomena and the infarcts. I think that we have ruled this out by our control studies. Thus, in a series of dogs with constrictions alone there were no infarcts. Secondly, in another series of controls we inserted rings, kept them there momentarily, and then removed them. Again, no infarcts were observed. These two series of control experiments lead us to believe that rheology probably doesn't cause infarcts. I hasten to add that those experiments in which we ligated or constricted the aorta without rings were conducted for a long period of time. Accordingly, if altered rheology did play a part, it had ample opportunity to do so. These preparations were maintained for as long as a week or so and we did not see infarcts.

With regard to your second question: the sterilization varied acording to the nature of the material. Most materials may be autoclaved, and whenever we can we autoclave our rings. Those which cannot be auotclaved are usually sterilized by ethylene oxide. When we sterilize with ethylene oxide, we carefully consult with a manufacturer to make sure that this procedure in itself will not alter the surface.

CHARLES MEALS, V.A. Hospital, Dallas, Texas: I was interested when you said the lining of your implant was benzalkonium. We're putting heparin lining in our tubing at Dallas now. We've been cross-linking it with heparin and glutar-aldehyde, and it seems to work very well being nonthrombogenic. Did you put that on these implants?

DR. KUSSEROW: The particular implants that I exhibited during the presentation were prepared according to the initial Gott technique. There is an absorbed lining of heparin upon the implant. It is apparently not as permanent as the heparin binding which one obtains with cross-linking or with covalent bonding. Accordingly, there is a very good possibility that the surfaces of which you speak would fare better in our test system.

DR. MEALS: Did you do any of the blood studies, for instance, like fibrinogen or any of the normal blood studies in comparison with this work?

DR. KUSSEROW: No, these are basically screening tests, and we have not done detailed studies of clotting factors, fibrinogen, and so forth. This should ultimately be done and probably would be done for any very promising test material.

QUESTIONER (Unidentified): Considering that the kidney is actively functioning during your studies, in any of your series had you assayed renal function for chemical substances or internal pressure flow changes as they may be associated with the infarct itself? Or did you do assays as an additional sensitive measure of the presence of your test samples? Did you do any renal function assays?

DR. KUSSEROW: No, we have not. This is a very interesting suggestion, and my answer is that we haven't.

TETSUZO AKUTSU, Univ. of Mississippi Medical Center, Jackson, Mississippi: How many

days did you leave the ring in the longest case?

DR. KUSSEROW: In the test system?

DR. AKUTSU: Yes.

DR. KUSSEROW: We arbitrarily terminate the implantation at five days. In some of the controls in which we performed simple constriction with no ring, implants were permitted to go for a week.

DR. AKUTSU: I think this method is a very good idea. We have been doing a total artificial heart implantation and in many cases surviving longer than one week after the experiment, we didn't see any thrombus formation in the device, but in others we saw the infarct in the kidney.

QUESTIONER (Unidentified): Did plasma hemoglobin play a role here at all, or any of the products of red cell lysis?

DR. KUSSEROW: The plasma hemoglobin did not appear to change appreciably. We have such a small surface area here that we could not detect alterations in plasma hemoglobin level. Your point is very important. I assume you refer to the release of factors such as ADP from lysed red cells which might, in fact, enhance the thrombotic propensity of the blood and tend to cause platelet aggregation or enhance it at the ring surface. We are in fact now initiating experiments to study the difference between the thrombotic propensity of a given test surface exposed to "normal" or non-traumatized blood and to blood which has been altered by hemolysis of traumatic origin.

SPONTANEOUS CARDIOPULMONARY DISEASE IN NONHUMAN PRIMATES: POTENTIAL MODELS

W. P. McNulty*

Some spontaneous diseases of the heart and lungs of nonhuman primates offer promise for exploitation as models for human diseases for which etiology, pathogenesis, prevention, and treatment are poorly understood. Most of these are "subclinical." This may be due to both the young average age of animals in primate colonies and to difficulties with accurate clinical observations. Generalized pulmonary arteriosclerosis and emphysema may accompany severe and prolonged infestation with pulmonary mites. Clinical syndromes and at least suggestive postmortem findings indicate that both sudden, unexpected death in infancy and hyaline membrane disease may occur in monkeys. Pneumoconioses due to a variety of materials occur with regularity. No spontaneous autoimmune disease, comparable to asthma, Goodpasture's syndrome, or Hamman-Rich disease, has been recognized. Primary pulmonary neoplasms have been very uncommon. Bacterial pneumonias account for most clinical pulmonary disease in conditioned monkeys; the most common organisms are enteric bacteria. Viral pneumonias are common in the first weeks after arrival in this country. Neither acute rheumatic myocarditis nor chronic rheumatic valvular disease has been reported, and the carrier rate and susceptibility of nonhuman primates to beta-hemolytic streptococcus is unknown. Autoimmune alterations of cardiac valves, such as may be seen in systemic lupus and rheumatoid arthritis, have not been observed. The valves do change with age, though calcification and functional impairment have not been described. Chronic focal myocarditis of unknown etiology is commonly present in several species of monkeys, but is not known to be of clinical significance. Coronary atherosclerosis is unusual except in animals fed highly atherogenic diets, although aging macaques do show concentric cellular intimal thickening in coronary arteries. Spontaneous cardiac anomalies occur but are rare. The major cardiopulmonary diseases of man become clinically significant late in life. Full use of nonhuman primates as models will require "geriatric colonies," i.e., maintenance of the animals for decades.

INTRODUCTION**

The use of nonhuman primates for medical research has disadvantages. They are expensive, to handle, and breed poorly, at least by comparison with established laboratory animals. But, as an item of faith, we who work with primates subscribe to the notion that in general the degree of similarity of disease in various species should correlate with evolutionary relatedness.

We seem to be on safe ground with respect to behavior and reproduction. The behavioral characteristics we share with monkeys and apes are obvious even at the zoo. Furthermore only monkeys and apes share with us the monthly cyclic physiologic and endocrinologic phenomena of the menstrual period in the female and the sustained satyriasis in the male.

In other anatomical and physiological systems a close similarity, at least from the point of view of pathogenesis of disease, is not obvious. The diseases of most interest for investigative medicine are those we know the least about: etiology, pathogenesis and natural history, and prevention, modification, and cure. Knowing little about these diseases, we cannot readily perform prospective investigations. We can, however, pay close attention to the spontaneous ailments in monkeys, in the hope that we may see more or less similarity to human disease in clinical syndromes and pathologic changes.

After nearly a decade of intensifying research with nonhuman primates, it is time to review the spontaneous diseases which have been found at autopsy. My remarks pertain only to the experience at the Oregon Regional Primate Research Center, and the interpretations are based solely on routine gross and light microscopic pathological examination.

RESULTS AND DISCUSSION

Approximately 4500 animals died since 1963; the majority of these were experimental sacri-

*Oregon Regional Primate Research Center Beaverton, Oregon 97005.

**Publication No. 571 of the Oregon Regional Primate Research Center. This study was supported by NIH Grant No. RR 00163 to the Oregon Regional Primate Research Center.

fices. Two thousand animals were examined postmortem, to the extent at least that organs were weighed and histological sections examined. Most of the remainder were inspected grossly by a pathologist and judged free of any obvious disease which was unrelated to the experiment which called for sacrifice. We have paid particular attention to those animals which died or were sacrificed for spontaneous disease, which died in quarantine, and which were born and raised here.

In America most serious human diseases, excluding accidents, occur early in life, or in late middle and old age. The age distribution of animals which have come to autopsy must therefore be taken into account in a survey for models. The lifespan of monkeys is not known with certainty. Most Old World species probably can live to 40 years or more; the span for New World species is believed to be less.

For animals born at the Oregon Center, the ages at autopsy, which included histologic examination of the heart and lungs, are given by species in Table I. The preponderance of infants reflects the many sacrifices for programs in fetal and neonatal physiology. Our own captive-born animals which are not used in these programs are considered especially valuable. They are still young and few have come to autopsy.

For imported, wild-born animals, time in residence is similarly shown in Table II. Many of these monkeys are either used in terminal experiments rather soon after arrival, or put in long-term projects, such as the breeding colony. Their true ages are of course not known, but most animals which survive the rigors of capture and importation appear young. Therefore even those animals which have been examined 2–8 years after arrival are probably not aging.

In summary we have for rhesus monkeys a good sample of material from the very young, a little from youth and early adulthood, and almost nothing from middle and old age. This should be kept in mind in the following survey.

Instead of the usual procedure of describing interesting postmortem findings in monkeys and relating them to a human condition, however common or clinically important it may be, I have reviewed our files for results, positive or negative, which can be grouped under a selected list of human cardiopulmonary diseases which are frequent clinical problems about which we wish we knew much more. The list is short and not intended to be exhaustive.

Pulmonary arteriosclerosis

Members of the genus *Macaca,* from Southeast Asia, are commonly infected with the mite *Pneumonyssus simicola,* which lives in the lumens of bronchi. The life cycle and means of transmission are not known. Adult imported animals are most heavily infested, but we have found the organisms in the lungs of young captive-born *Macaca mulatta* monkeys which have lived in groups with older animals.[1]

Characteristically a focus of bronchiectasis is present at infected sites; the bronchial smooth

TABLE I.—*Heart/Lung Examinations, Captive-born Primates, Age in years at autopsy*

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10	Total
Macaca fascicularis	3	2	0	0	0	0	0	0	0	0	0	5
Macaca niger	8	2	2	0	0	1	0	0	0	0	0	13
Macaca fuscata	3	0	0	0	0	0	0	0	0	0	0	3
Macaca nemestrina	10	5	2	1	1	0	1	0	0	0	0	20
Macaca mulatta	283	45	12	8	3	3	0	0	0	0	0	354

TABLE II.—*Heart/Lung Examinations, Imported Primates, Years in residence at autopsy*

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10	Total
Alouatta caraya	12	0	0	2	0	0	0	0	0	0	0	14
Saimiri sciurea	101	17	12	0	0	0	0	0	0	0	0	130
Macaca fascicularis	13	0	0	0	0	0	0	0	0	0	0	13
Macaca niger	11	0	0	0	0	0	0	2	0	0	0	13
Macaca speciosa	3	2	3	1	0	0	1	0	0	0	0	10
Macaca fuscata	3	2	3	2	1	0	0	0	0	0	0	11
Macaca nemestrina	12	5	4	6	4	0	3	0	0	1	0	35
Macaca mulatta	185	47	66	41	22	20	16	10	5	0	1	413

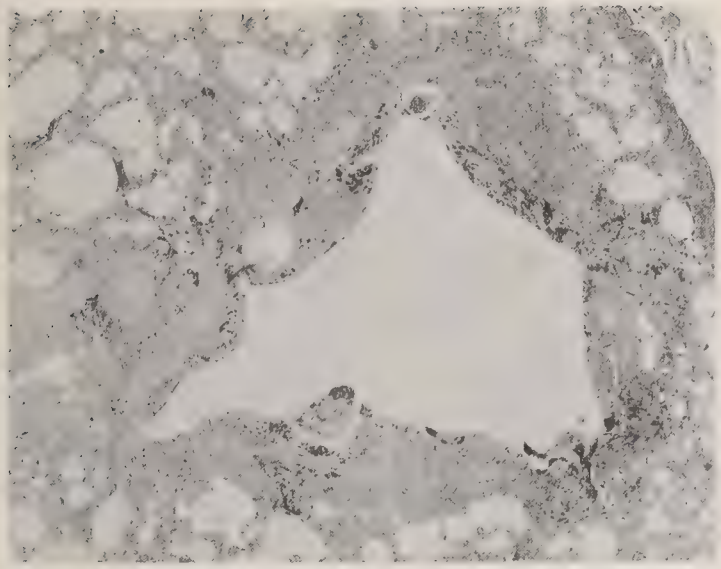


FIGURE 1.—Mite infection in a male rhesus monkey. Sections of mites may be seen in a dilated bronchus, showing muscular hypertrophy and adjacent chronic inflammation and pigment deposition. H & E, 31x.



FIGURE 2.—Large branch of pulmonary artery in male rhesus monkey infected with mites. The vessel is tortuous and thickened; the elastic lamellae are fragmented. Acid orcein Verhoeff, 60x.

muscle is hypertrophic, and in the peribronchial connective tissue and adjoining alveoli are dense accumulations of lymphocytes, histiocytes, and eosinophils. Pigment of at least two kinds is present, one of which we believe is a hemoglobin-derived fecal discharge from the mites, the other fragments of chitin (Fig. 1).

Of great interest are associated changes in pulmonary arteries, which also seem to be of two kinds. Those arteries immediately adjacent to mite lesions are markedly irregular; the intima is erratically thickened and the elastic fibers in the media are fragmented and distorted (Fig. 2). These changes seem to be caused directly by the mites, and Woodard[2] has described mites apparently lodged in or migrating through pulmonary arterial walls.

In severely affected animals all pulmonary arteries are tortuous and heavy-walled with thickened intima. This is true for vessels at a distance from the parasites (Figs. 3, 4); even the main pulmonary artery may be involved (Fig. 5). By gross inspection we have recognized right ventricular hypertrophy in only one case, and we have just begun to collect ratios of the weights of the right and left ventricular walls.

We suspect that pulmonary hypertension is present and suggest this may be a useful model for exploration. The animals appear clinically unaffected except for a cough, but the severity of infection may be estimated in life by radiographs of the chest. To date no physiologic measurements have been made of pulmonary vascular and ventilatory function.

Pulmonary emphysema

Emphysema may be systematically studied and classified as to anatomical type only by



FIGURE 3.—Cut surface of fixed lung, male rhesus monkey infected with mites. Thickened pulmonary arteries are prominent.

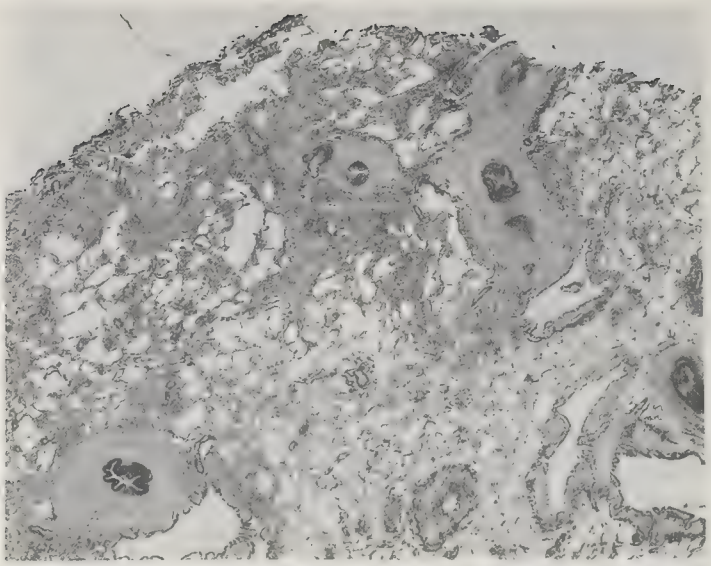


FIGURE 4.—Photomicrograph of lung in Figure 3. Massive muscular hypertrophy is present in every pulmonary artery. Acid orcein Verhoeff, 11.5x.



FIGURE 6.—Closer view of another cut surface of lung from case shown in Figure 3. The air spaces are raggedly enlarged.

special techniques not normally used in routine autopsies. The lungs must be gently inflated with formalin or formalin vapors before cutting, and large thick sections are desirable. We have not used these techniques, and our 12 diagnoses of emphysema have been based on gross observations of large doughy lungs, sometimes with bullous cysts, or on microscopic evidence of dilation of terminal air spaces and destruction of alveolar walls. Classification has not been attempted.

Emphysema occurs in association with severe infestation with mites (Figs. 6, 7), but neither anatomical classification nor correlation with severity of parasitism or degree of arterial change has been made. Since the pathogenesis of the several kinds of pulmonary emphysema is controversial, macaques bearing lung mites



FIGURE 5.—Main pulmonary artery from lung in Figure 3. The intima is uniformly thickened and contains numerous small elastic fibers. Acid orcein Verhoeff, 42x.

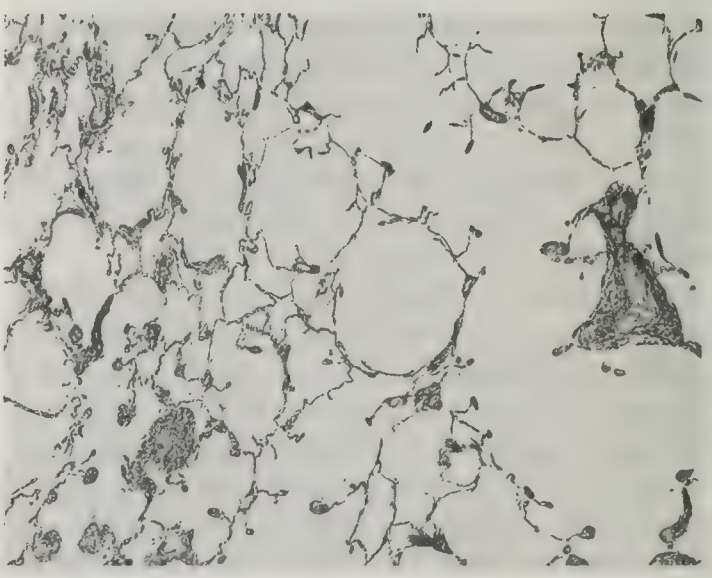


FIGURE 7.—Photomicrograph of lung in case shown in Figures 3 and 6. The air spaces are enlarged and simplified. Stubs of alveolar walls have terminal clubs of smooth muscle. Acid orcein Verhoeff, 50x.

deserve more detailed pathologic examination and correlation with functional tests.

Sudden unexpected death in infancy

"Crib death" is a frequent diagnosis in human infants dying in the second through the twelfth month of life. This is the circumstance which in the past has been called "status thymolyphaticus," "smothering in the bed clothes," or "early interstitial pneumonia." Typically a child in outward good health in the evening is found dead in the morning. No consistent anatomical changes are found at autopsy, and bacteriologic and virologic studies of lungs and blood have been unrewarding. The condition remains a tragic enigma.

We have made this diagnosis in 4 infant rhesus monkeys for obvious reasons on clinical rather than anatomical grounds. Since by definition the "illness" has no known natural history and death comes without warning, the occurrence of this condition does not constitute a model in the usual sense.

Hyaline membrane disease

This affliction is poorly named, because if death occurs very early the characteristic eosinophilic precipitates in respiratory and terminal bronchioles may be difficult to find. The diagnosis may usually be made with confidence, however, from a characteristic pattern of dilatation of respiratory bronchioles and collapse of alveoli.[3] Twice this has been tentatively diagnosed in rhesus babies; the pattern was present but not the membranes (Fig. 8).

In human infants, immaturity and asphyxia appear to be predisposing factors, leading to a failure in maintenance of the concentrations of lipid surfactant in alveoli necessary for aeration.[4] These factors might be easily manipulated experimentally.

Pneumoconioses

Deposits of particulate foreign material are common in lungs. Kaolin (aluminum silicate) has been inadvertently put in the lungs during attempted gastric intubation, resulting in a granulomatous pneumonia. The pigments of mite infestation have already been mentioned. Unidentified brown and black pigments, sometimes crystalline and polarizing, appear often in alveolar macrophages and in interstitial tissue and regional lymph nodes. Presumably some of this is anthracotic pigment from suburban air; some may be from dusts and aerosols peculiar to colony environments. In no case has this deposition been associated with emphysema, fibrosis, or pulmonary arteriosclerosis (except in the case of mites) and we have not tried to identify the substances.

Immune diseases

Some of the least understood human pulmonary diseases are loosely grouped as inappropriate responses of the immune system, such as bronchial asthma, Goodpasture's syndrome, rheumatic pneumonitis, and progressive pulmonary sclerosis (Hamman-Rich disease). We have not seen, or at least recognized, changes in monkey lungs suggestive of these conditions.

Neoplasms

Although some major factors in the etiology of human lung cancer are well known (cigarette smoking, radiation, asbestos) possible other contributing factors, such as occult viruses and inheritance, are not. With the exception of a

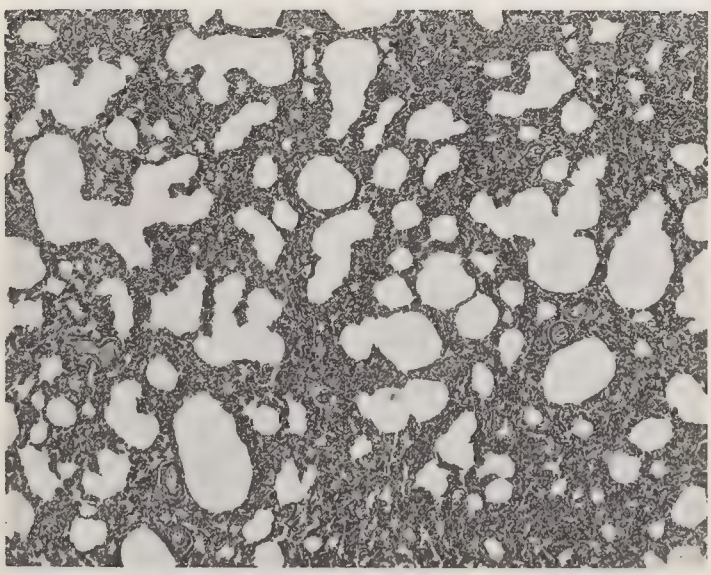


FIGURE 8.—Lung of a female rhesus infant dying with respiratory distress in first day of life. The terminal bronchioles are dilated, alveoli are collapsed. Hyaline membranes are not present. H & E, 50x.

single questionable alveolar cell carcinoma[5] in a wild-shot howler monkey *(Alouatta caraya)* (Fig. 9), we have seen no primary pulmonary neoplasms. One case of widespread metastatic malignancy of the lungs in a pregnant rhesus monkey was examined (Fig. 10); the presumptive source was a choriocarcinoma, but a primary lesion in the uterus was not found.

Infectious disease

Bacterial pneumonias have accounted for most acute pulmonary disease. Cases occur chiefly during initial quarantine, with mortality sometimes reaching 10% in winter months, or as complications of surgery or unrelated diseases. Our animals are all "conditioned" in an importer's facility for 2 to 3 months before delivery to the Primate Center. Hence we do not see the viral pneumonias, especially measles, which are said to be frequent in newly imported animals. The agents commonly isolated from pneumonias are enteric organisms: Friedlander's bacilli, *Proteus* species, and *E. coli*. Coccal pneumonias are rare. In this respect monkeys resemble very young, or aged, or debilitated people, although the monkeys are often none of these. This may reflect a fundamental species difference in defenses against bacteria, or merely a lack of concern by the monkeys for sanitation (the flora of the throat in well animals is dominantly enteric organisms).

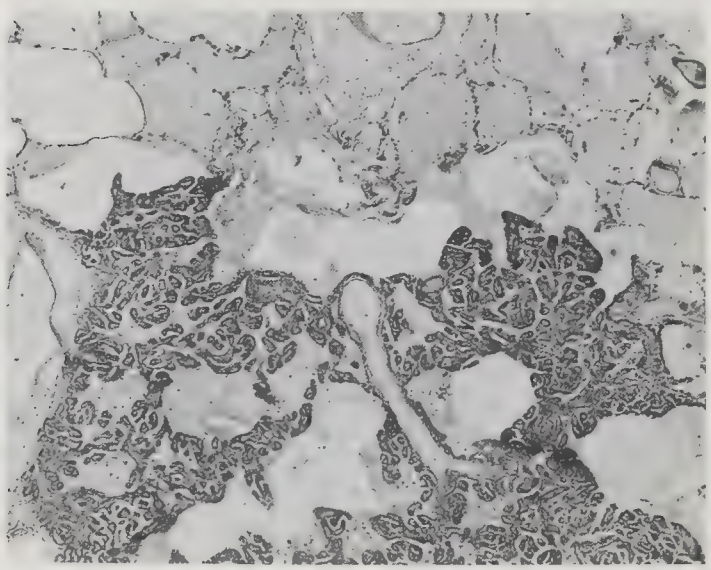


FIGURE 9.—Margin of a solitary epithelial lesion (alveolar cell carcinoma?) in the lung of a male howler monkey. PAS, 50x.



FIGURE 10.—Multiple metastases (probably choriocarcinoma) in the lungs of a pregnant rhesus monkey.

Bacterial pneumonias are usually focal or confluent focal, rather than lobar. Friedlander's pneumonias are prominently necrotizing and hemorrhagic.

Rheumatic fever

Nothing resembling acute rheumatic myocarditis or chronic rheumatic valvulitis has been found. Clinical illness resembling streptococcal pharyngitis has not been seen, and beta hemolytic streptococcus has been isolated only 8 times in cultures from throat, blood, lungs and skin lesions. The prevalence of a pharyngeal carrier state for beta hemolytic streptococcus is unknown and our laboratory has not screened sera for streptococcal antibodies.

Descriptions of other valvular deformities, such as may occur in systemic lupus erythematosus and rheumatoid arthritis, are absent from

our files. The leaflets of cardiac valves are said to thicken with age, microscopically showing an increase in loose connective tissue with minimal inflammatory infiltrate.[6] We have not systematically examined the valves and cannot comment on this observation. At any rate, we have not seen calcification or deformities or associated signs of functional impairment in valves in the older of our animals.

Myocarditis

The myocardium of most macaques shows scattered small foci of accumulations of lymphocytes, histiocytes, and Anitschow myocytes (Fig. 11). Occasionally in these foci are a few necrotic muscle fibers. The cause and significance of these lesions is unknown. Necrosis cannot be a prominent feature, since focal scarring is uncommon. There is no associated cardiac decompensation or hypertrophy, and the coronary arteries are normal or show minimal intimal thickening.

The lymphocytic infiltrate brings viral infection to mind, but the prevalence, chronicity and focal distribution of the lesions weigh against this suggestion. Parasites have been looked for without success.

The foci are faintly reminiscent of Aschoff bodies, and it is tempting to suspect an immune process. However, the ubiquity of the condition and apparent lack of clinical significance are discouraging. Still, exploration with immunopathologic techniques might be fruitful.

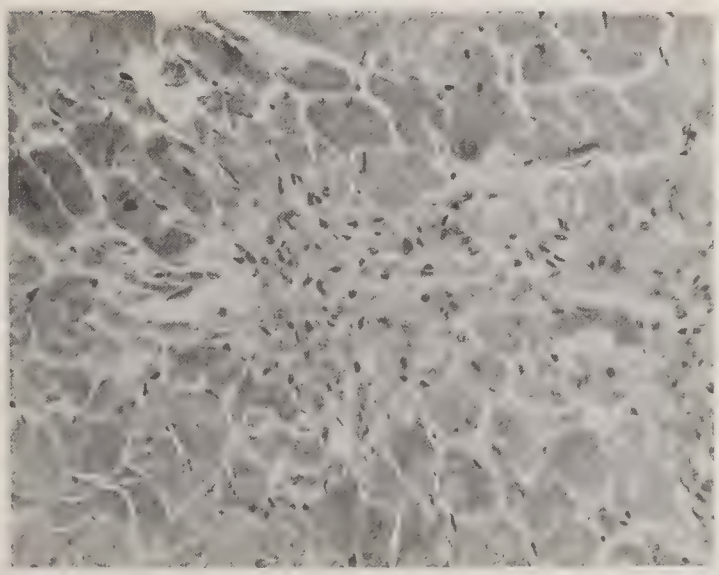


FIGURE 11.—Focal chronic myocarditis in an adult male rhesus monkey. H & E, 250x.

Hypertension

Routine measurement of arterial blood pressure is not practical in large numbers of monkeys and so the incidence of clinical hypertension, or for that matter even the normal range of tension in unanesthetized animals, is not known. However, our postmortem records do not suggest that we are missing something.

The hearts of 350 rhesus monkeys averaged from 0.6% of body weight at birth to 0.4% at maturity. The hearts of 20 of these weighed more than half again as much as expected, but none showed microscopic hypertrophy of myocardial fibers or interstitial scarring. No monkey had hypertensive arteriolopathy, and 11 of the 20 were pregnant or had suffered recent weight loss. Hypertrophy of myocardial fibers has been described in chronically hypertensive rhesus monkeys,[6] but measurements of weight and size of the heart were not given.

Atherosclerosis

This has been successfully induced by diet in several species of New and Old World monkeys.[7] These diets, from the monkey's point of view, are bizarre; they contain high concentrations of cholesterol or lipids or both, concentrations much higher than those in laboratory chows or natural foods. The morphology of the lesions produced resembles that of the disease in man, especially in Old World monkeys and most particularly in chimpanzees.

The rate of induction of experimental atherosclerosis is much faster than the development of spontaneous human disease. This characteristic is enforced by the short span of investigators' attention and short duration of experimental projects, short, that is, with respect to the lifespan of monkeys. Two important questions therefore require answers: (1) Is diet-induced atherosclerosis an acceleration and accentuation of the same process which would occur spontaneously, with much individual variation, on a "normal" diet? (2) If so, are methods which may be found to modify the evolution of rapidly

developing diet-induced atherosclerosis applicable to the more leisurely course of spontaneous disease?

A partial answer to the first question may of course be found in surveys of the vessels of animals which have reached middle and old age on diets which are not bizarre.[7] But this answer is not easy to attain. Wild-caught animals are always of uncertain age, and probably only a few reach anything near their potential lifespan. Breeding of captive animals has been achieved on a significant scale only in the last few years, and the pressure for terminal experimental use and expense of maintenance will ensure that few captive-born animals will live out a life of the order of 40 years.

Furthermore, wild diets and inexpensive laboratory chows probably differ, in the opposite direction with respect to fat and cholesterol, from the average Western human diet as much as do laboratory atherogenic diets.

A partial answer to the first question which may be drawn from our own files is equivocal. We have not seen a single instance of damage to heart, brain or kidneys which could be attributed to atherosclerotic vascular insufficiency. However, the aorta and renal and coronary arteries do show cellular intimal thickening which increases with age (Fig. 12). The internal elastic lamella becomes fragmented and duplicated, but foam cells, lipid deposits, necrosis and calcification have not occurred. We can therefore conclude, from a very small sample, that rhesus monkeys do in time develop arterial intimal thickening similar in some respects to that seen in human vessels. The documentation of the full natural history of this thickening will require many years and many animals. We have so far had the opportunity to examine only 8 postpubertal rhesus monkeys of known age, the oldest of which was 14 and the rest 7 or less!

As Dr. Stout has indicated,[8] case reports of clinically significant atherosclerosis in zoo primates will remain for some time our source of confidence that monkeys and apes are appropriate models.

The answer to the second question about modifying the course of spontaneous atherosclerosis lies too far in the future for consideration here.

Congenital cardiac anomalies

The utility of congenital heart disease in experimental animals as a model lies rather more in identification of causes rather than documentation of anatomical variations and experimental manipulations of the disordered dynamics of flow. Malformations do occur in nonhuman primates, but descriptions of only eleven naturally occurring cases have been published, all unexpected findings at postmortem.[7] Wild animals with clinically important anomalies probably do not survive long, and more cases will doubtless be found as the population of captive-born animals increases.

At the Oregon Center only one spontaneous anomaly has been noted. A wild-born Japanese macaque *(Macaca fuscata)*, 6 years in residence and the mother of 2 infants, recently died in severe right heart failure. She had almost no interatrial septum (failure of the septum primum) (Fig. 13). An enlarged heart had been noted radiographically 6 years before death. There was nothing in a routine autopsy to suggest a generalized genetic syndrome, but no biochemical or chromosomal studies were made. The two children are fortunately available for further observation.



FIGURE 12.—Intimal thickening, with elastosis, in the aorta of an adult female rhesus monkey. Verhoeff-van Gieson, 50x.



FIGURE 13.—Cardiac anomaly in adult female Japanese macaque monkey. The interatrial septum is nearly completely absent. View from above through opened unilocular atrium, showing free upper margin of interventricular septum, tricuspid valve to the right, mitral valve to the left.

SUMMARY

Nonhuman primates have not so far proven to be fertile source of models for poorly understood human cardiopulmonary disease, at least at the Oregon Primate Center. Pulmonary arteriosclerosis and sometimes emphysema are seen in the lungs of macaques heavily infested with mites. Crib death and hyaline membrane disease have been tentatively diagnosed on a few occasions. We have seen no immunopathological pulmonary diseases and only one doubtful primary malignancy in the lung. Bacterial pneumonias are common, but of no special interest as models.

We have not seen immunopathologic heart disease. Atheroscelerosis, or at least arterial intimal non-lipid thickening, occurs with age but has been of no clinical significance. One cardiac congenital anomaly was found.

These results are somewhat disappointing, but perhaps not surprising. The population of animals we have been surveying is young. Cardiopulmonary disease is uncommon enough in young people that even if the incidence were similar in monkeys and men we could have seen but very few cases in our series—as is the case. On the other hand, cardiopulmonary disease is almost the rule in old people, but we have had no old monkeys at which to look.

Furthermore, we are not likely even in the future to have many aging animals to examine unless the pattern of support for primate research is modified to permit maintenance and observation of captive-born animals for periods of time so long that before most of the data is collected, most of the beginning investigators will be dead or too atherosclerotic themselves to care any more. Such support would be enormously expensive and would require continuity of protocol and record-keeping over a period of time quite foreign to our usual ideas about biological research.

Is it worth knowing, for example, that truly old monkeys, inactive captives and over-eaters, have coronaries, strokes and arteriosclerotic renal failure? If they do, is it worth the long wait before carefully controlled prospective investigations of the effects of exercise, diet and heredity might point with assurance to methods of reducing or preventing atherosclerosis in man? Or, from a practical point of view are we our own best experimental animals for study of the diseases considered here?

It appears unlikely that an answer can be obtained from the first 9 years of postmortem findings in the population of experimental animals at one large primate center.

ACKNOWLEDGMENTS

Most of the postmortem examinations were performed by colleagues Dr. A. Knezevich, Dr. C. Maruffo, Dr. J. Palotay, and Dr. H. Uno.

REFERENCES

1. KNEZEVICH, A. L., and MCNULTY, W. P., JR.: Pulmonary acariasis *(Pneumonyssus simicola)* in colony-bred *Macaca mulatta*. J. Am. Vet. Med. Assoc., 20:693–696, 1970.
2. WOODARD, J. C.: Acarous *(Pneumonyssus simicola)* arteritis in rhesus monkeys. J. Am. Vet. Med. Assoc., 153:905–909, 1968.
3. GRUENWALD, P.: The significance of pulmonary hyaline membranes in newborn infants. J. Am. Med. Assoc., 166:621–623, 1958.
4. REYNOLDS, E. O. R.: Hyaline membrane disease. Am. J. Obstet. Gynec., 106:780–797, 1970.
5. MARUFFO, C. A.: Spontaneous tumours in howler monkeys. Nature, 213:521, 1967.

6. LAPIN, B. A., and YAKOVLEVA, L. A.: *Comparative Pathology in Monkeys*, pp. 132–176 (C. C. Thomas, Springfield, Illinois, 1963).
7. MCNULTY, W. P., and MALINOW, M. R.: The Cardiovascular System, in *Pathology of Simian Primates*, ed. R. N. Fiennes, pp. 756–808 (S Karger, Basel, Switzerland 1972).
8. STOUT, C., and BOHORQUEZ, F.: Arteriosclerosis and other vascular diseases in zoo and laboratory animals. These proceedings.

DISCUSSION

W. A. THOMAS, Albany Medical Center: I'd just like to predict that if you do this 50- or 100-year study in your primates with monkey chow, or any similar low-fat, low cholesterol, high carbohydrate diet, you're not going to get much disease in the arteries. Similarly, there won't be any clinical symptoms. There have been many studies of human populations, autopsies and otherwise, subsisting for a lifetime on similar diets. Out of thousands of autopsies in East Africa for example, there have been no strokes from arteriosclerosis and no myocardial infarcts. There has been some disease, but no clinical symptoms.

DR. MCNULTY: I agree completely. I should have stated that it was implicit anyway in this 50-year study that they must have bacon and eggs for breakfast everyday.

J. MOOR-JANKOWSKI, New York University Medical Center: I want to make two comments. First, Dr. McNulty stated a few times that these animals are expensive, that it's worthwhile to work with them, but that they are more expensive than other animals. We have heard this very often. Those of you who will be here at the Sunday morning session will hear that in New York City today it costs more to keep a dog for one year than it costs to keep a monkey. This includes the purchase price. The purchase price for a carefully bred dog in Europe today is equivalent to the price for which we can breed an untamed monkey in New York. This includes, as you know, very high labor costs. So the time is very rapidly approaching that monkeys will not be more expensive than other standard experimental animals.

One more comment, speaking of streptococcal pharyngitis, it has been induced experimentally in the baboon and it occurs spontaneously in chimpanzees with antibody levels similar to those of man. This is the work by a group from Toronto carried out in our laboratory.

DR. MCNULTY: We have no baboons or chimpanzees.

WILLIAM R. MURPHY, University of Minnesota, Minneapolis: We've been worried by the presence of a very high infectivity rate with mites in the baboons with which we work. Sometimes a shipment will be very highly infested. Other shipments will be completely clean, and we have no way of detecting the presence of the mite until we actually do the experiment. We often have to abort it. Could you comment?

DR. MCNULTY: Yes. Our radiologist feels that he now, after experience of several years, can diagnose moderate to heavy degrees of infestation of mites on chest films. It gives a characteristic mottled appearance. He confused this earlier with tuberculosis, but he feels now that he can distinguish it quite clearly. Other than that, I know of no way of diagnosis. Sputum examination, if you could get it, is a possibility. The larva forms, or young forms, have been reported in stools. We have not looked for them, however.

DR. MURPHY: Are you familiar with any way of treating them?

DR. MCNULTY: Yes. I've heard of ways of treating them with organic phosphate compounds which, while they do not cure the disease, at least prevent its increase and probably its spread. This way new born animals may be kept from becoming infected. The young in association with groups of their elders can become infected; Young monkeys which are kept separate do not. There's another problem of ever getting them to breed again, too. But this is one preventitive treatment—isolation between generations.

DR. MURPHY: Is this disease of any danger to the animal handlers?

DR. MCNULTY: We don't think so. Mites in the human sputum have been reported from Southeast Asia but they appear to be different species. I don't know of any clinical disease associated with it. It should be borne in mind by anyone who is contemplating any cardiovascu-

lar or particularly pulmonary, physiologic experiments. There are disturbances in function, even though we don't clinically recognize them.

ALBERT M. JONAS, Yale University School of Medicine, New Haven: Just a comment on the lesions of the pulmonary vessels that you're seeing. There is some danger obviously in relating these lesions to pulmonary hypertension. If we want to compare them to some of the lesions that we see in cats, in which the lesions of the pulmonary hypertrophy and hyperplasia can even be more florid than what you're seeing here, then these animals are hypertensive but many of them are not. If we say these monkeys have pulmonary hypertension, it might be just a little bit premature at this point. The other point I'd like to make is that if the *Pneumonyssus* is a very common parasite, then it's difficult to relate this in terms of causality to the lesion. It may in fact be related, but we have to be a little careful about that also.

DR. MCNULTY: I really hoped I was presenting this paper to outline questions worthy of investigation rather than to provide answers. I agree with most of the things you said. The degree of arterial change, I think, has pretty clearly correlated with the extent of evidence of mite infestation. But other than this associative kind of evidence, we have none. There is no sure way to diagnose hypertension except to measure the pressure and this has not been done.

ARTERIOSCLEROSIS AND OTHER VASCULAR DISEASES IN ZOO AND LABORATORY ANIMALS

C. Stout and F. Bohorquez*

The material to be presented was gathered during a five-year period from autopsies on animals dying in the Oklahoma City Zoo and the psychological research laboratory of Dr. W. B. Lemmon at the University of Oklahoma. Aortas and epicardial coronary arteries from large hearts were removed, flattened on cardboard, stained with Sudan IV and graded for intimal involvement using a modification of the method originally described by Holman. Contiguous microscopic sections of the various arterial lesions were stained with Oil Red O, H & E and Weigert's elastic stain. Typical atherosclerotic lesions were found in many of the birds, and smaller atherosclerotic lesions were found in a variety of other mammals, including anteaters, aardvarks and Zebu cattle. Atherosclerotic lesions were also common in nonhuman primates, particularly squirrel monkeys and chimpanzees. Chimpanzees were the only animals in which significant atherosclerotic lesions were present in the large coronary and cerebral arteries. None of these lesions were of clinical importance, however. Proliferative intimal lesions consisting primarily of smooth muscle cells were found in almost every species examined. In seals and sea lions, these lesions were distributed in the aorta in a fashion similar to that seen in atherosclerosis in man. It was interesting that no lipid was present in the lesions of the seals and sea lions, particularly since lipid insudation is thought to stimulate smooth muscle cell proliferation in the early stages of human atherosclerosis. It was also interesting that mural thrombi were almost never seen.

INTRODUCTION**

Studies of spontaneous vascular lesions in animals are important for several reasons. Conducted in a particular species, such as the work of Clarkson in pigeons,[1] Middleton in squirrel monkeys,[2] McGill in baboons,[3] Moreland in miniature swine,[4] Malinow in howler monkeys,[5] and others,[6] they provide baseline data for comparison with experimentally induced arterial lesions in that species. Conducted in a large number of species, such as the work of Fox[7] and Ratcliffe[8] at the Philadelphia Zoo, Vastesaeger[9] at the Antwerp Zoo, Finlayson[10] at the London Zoo, and others,[6,11,12] they provide a panorama of the types of lesions which may occur in the animal kingdom. Studies of the latter type may also identify animals or diseases which are suitable for more intensive investigation, the so-called animal models. A list of such animal models has recently been compiled by Cornelius.[13] Most importantly, studies of animals in natural or captive surroundings may provide insights into mechanisms operative in health and disease. Examples of the latter would include the association of adequate nutrition with the decreasing incidence of death from infectious disease, and the association of social stress with the increasing incidence of myocardial fibrosis and stenosis of the small intramyocardial arteries in mammals and birds in the Philadelphia Zoo.[14]

The studies to be reported in this communication were done between 1964 and 1968 at the Oklahoma City Zoo. From over 500 autopsies, a variety of arterial intimal and medial lesions have been selected for presentation. In addition, two animal models will be described, one with morphologic changes similar to those in preeclampsia in humans, and the other with morphologic changes similar to those in essential hypertension in humans.

MATERIALS AND METHODS

In 1964, the University of Oklahoma Health Sciences Center opened the Institute for Comparative Pathology in conjunction with, and on the grounds of the Oklahoma City Zoo. The laboratory was remodeled for pathologic studies with funds from the National Heart Institute (HE 08725) and the Oklahoma Zoological So-

* Department of Pathology, University of Oklahoma Health Sciences Center, 800 N. E. 13th Street, P. O. Box 26901, Oklahoma City, Oklahoma 73190.

** Supported by Grant HE 08725 from the National Heart Institute, U.S. Public Health Service.

ciety. The purpose of the project was to study heart disease in zoo animals.

The material to be presented here was derived from the study of 405 aortas from 162 species. Autopsies were consecutive for the most part, but all aortas were not suitable for study. Complete gross autopsies were done on all individuals, with histologic examination of most organs and tissues in approximately one-half of the cases. Clinical records at the zoo were relatively good. Many animals died within a few weeks or months after acquisition, however, so that correlation of clinical and pathologic data was not always possible.

Aortas and epicardial coronary arteries from large hearts were opened longitudinally, flattened on cardboard and fixed in 10% formalin. After fixation, the vessels were stained with Sudan IV in isopropyl alcohol, using a modification[15] of the method originally described by Holman et al.[16] The extent of intimal involvement with fatty streaks, fibrous plaques and/or complicated lesions was then graded independently by two observers and expressed as a percentage of the total intimal area. Representative blocks of arterial lesions were then sectioned on a cryostat and stained with Oil Red-O-hematoxylin. The blocks were next embedded in paraffin and contiguous histologic sections were cut and stained with H & E and Weigert's elastic stain. Other stains were employed as indicated. Portions of this work have been reported elsewhere.[17-22]

RESULTS

ARTERIOSCLEROSIS

Aortic Lesions

Fatty Streaks. Fatty streaks were the most common lesions seen, being present in 61 (51%) of 121 hoofed mammals, 60 (58%) of 104 nonhuman primates, 7 (13%) of 54 carvivores, none of 12 pinnipeds, 62 (86%) of 72 birds, and 11 (26%) of 42 exotic mammals (Table I). Exotic mammals were arbitrarily defined as those from the orders Insectivora, Edentata, Rodentia, Marsupialia, Hyracoidea and Tubulidentata. Fatty streaks were noted in all portions of the aortas, but tended to occur more frequently in the thoracic segments. Less than 5% of the total surface of the aorta was involved in most of the individuals. Notable exceptions were a three year old male chimpanzee *(Pan troglodytes)* with 20%, a squirrel monkey *(Saimiri sciureus)* with 40%, a domestic turkey with 80%, a common duck with 50%, and 2 ostriches *(Struthio camelus)* with 60% and 65%, respectively (Tables III-VII). Microscopically, these lesions consisted of focal deposits of Oil Red O positive material within a normal or slightly thickened intima. Intimal thickening, when present, was comprised primarily of smooth muscle cells. The lipid droplets were usually small and evenly dispersed, although typical foam cells were seen occasionally, usually in birds or non-human primates. Many of the finely dispersed lipid droplets appeared to be extracellularly located, although this determination was not always easily made because of suboptimal preservation of tissues. The lipid droplets tended to cluster along the intimal surface of the internal elastic membrane in the abdominal aorta, while in the thoracic aorta they often extended into the inner media (Figures 1 and 2).

TABLE 1.—*Prevalence of Aortic Lesions in Mammals and Birds*

		Number of Individuals				
Species Group	Total	With Lesions	Fatty Steaks	Fibrous Plaques	Atheromatous Plaques	Other*
Hoofed mammals	121	82	61	57	1	20
Non-human primates	104	63	60	19	2	0
Carnivores	54	19	7	19	0	6
Pinnipeds	12	11	0	11	0	0
Birds	72	65	62	42	17	6
Exotic mammals	42	16	11	8	4	3
TOTALS	405	256	201	156	24	35

* Medial degenerative and parasitic lesions

TABLE II.—*Prevalence of Coronary Artery Lesions in Large Mammals and Birds*

		Number of Individuals			
Species Group	Total	With Lesions	Fatty Steaks	Fibrous Plaques	Atheromatous plaques
Hoofed mammals	38	12	2	12	0
Non-human primates	17	5	3	3	0
Carnivores	10	2	1	1	0
Pinnipeds	8	0	0	0	0
Birds	7	4	4	3	0
Exotic mammals	5	3	2	2	0
TOTALS	85	26	12	21	0

TABLE III.—*Taxonomic Classification and Prevalence of Arterial Lesions in Hoofed Mammals*

			Number of Animals			% of Aorta Containing Lesions		
Order	Common Name	Scientific Name	Total	With Lesions	With Atheroma	Fatty Steaks	Fibrous Plaques	Other
Perissodactyla	Donkey	Equus asinus	5	1		1	tr	
	Zebra	Equus burchelli	2	1		tr	tr	
	Tapir	Tapirus sp	1	1			tr	
Artiodactyla	Alpaca	Lama pacos	1	1		tr		
	Arabian camel	Camelus dromedarius	2	2		2	1	
	Giraffe	Giraffa camelopardalis	1	1		1	tr	
	Pronghorn	Antilocapra americana	5	2		tr	tr	
	Axis deer	Axis axis	1	1			1	
	Barasingha	Cervus duvauceli	2					
	Wapiti	Cervus canadensis	7	5		tr, tr, 2, 2, 5	tr, 1, 2, 5	+, +, +, +
	Reindeer	Rangifer tarandus	2	1			tr	+
	Mule deer	Odocoileus hemionus	9	7		tr, tr, tr, 1, 1, 1	1, 1, 2, 2	+, +, +
	White-tail deer	Odocoileus virginianus	7	6		1, 1, 1, 4, 10	1, 2, 5	+, +
	Muntjac	Muntiacus muntjak	1	1		1	5	
	Zebu	Bos indicus	4	2	1	10	tr, 3	
	American bison	Bison bison	1	1		tr	tr	
	Sitatunga	Tragelaphus spekei	7	4		tr, 1	tr, tr, 5, 10	
	Greater kudu	Tragelaphus strepsiceros	6	6		tr, tr, tr, tr, 1	tr, tr, tr, 1, 3	
	Nyala	Tragelaphus angasi	2	2		tr	tr	
	Nubian ibex	Capra ibex	3	2		tr, 2	tr, 1	
	Markhor goat	Capra falconeri	4	2		tr, 2	2	
	Angora goat	Capra hircus	5	4		tr, tr, 1, 2	tr, 1, 2	
	Argali	Ovis ammon	1	1		tr	10	
	Cape hartebeest	Alcelaphus buselaphus	1	1		1		
	Sable antelope	Hippotragus niger	2	1			1	+
	Kob	Kobus Kob	5	3		1, 2	tr, tr	+
	Defassa waterbuck	Kobus defassa	2	2		1, 15		
	Addax	Addax nasomaculatus	3	2		tr, tr		
	Topi	Damaliscus korrigum	2	2		tr, tr	tr	
	Grant's gazelle	Gazella granti	11	10		tr, tr, tr, tr, 1	tr,tr,tr,tr,tr,tr,tr	+,+,+,+,+,+,+,+
	Blackbuck	Antilope cervicapra	10	3		tr	tr, tr, 3	+
	Gemsbok	Oryx gazella	2	1		tr	tr	
	Persian gazelle	Gazella subgutturosa	2	1		tr	tr	
	African guinea hog	Potamochoerus porcus	2	2		tr, tr	tr	
		TOTALS	121	82	1	61	57	20
		%		68%	1%	50%	47%	16%

+ = Medial degeneration and/or calcification
tr = Trace

Fibrous (Myoelastic) Plaques. Fibrous plaques were found in 57 (47%) of 121 hoofed mammals, 19 (18%) of 104 non-human primates, 19 (35%) of 54 carvivores, 11 (92%) of 12 pinnipeds, 42 (58%) of 72 birds, and 8 (19%) of 42 exotic mammals. (Table I) Fibrous plaques were located primarily in the abdominal segments of the aortas, frequently at the iliac bifurcation or trifurcation. From traces to 3% of the intimal surface of the aortas contained fibrous plaques in most of the individuals. Exceptions were an elderly male rhesus monkey *(Macaca mulatta)* with 15%, 3 peacocks *(Pavo cristatus)* with 10%, 12%, and 15%, respectively, a domestic turkey with 10%, a Goliath heron *(Ardea goliath)* with 15%, an argali *(Ovis ammon)* with 10%, and 3 California sea lions *(Zalophus californianus)* with 10%, 10%, and 11% respectively (Tables III-VII). Microscopically, the lesions were composed of smooth muscle and elastic tissue elements. In larger plaques, the smooth muscle cells of the lesions were sometimes oriented in different planes. Most commonly, the cells nearest the media were oriented longitudinally or obliquely, while those adjacent to the lumen were oriented circumferentially. The internal elastic lamella was usually intact beneath small lesions and fragmented or absent beneath larger ones. Many fibrous plaques contained lipid droplets, which were usually distributed throughout the thickness of the lesions, but were most dense at the outer edges. Foam cells and central accumulations of lipid were seen primarily in the birds and non-human primates (Figures 3 and 4).

Atherosclerotic Plaques: Atherosclerotic plaques were found in 1 (1%) of 121 hoofed

TABLE IV.—*Taxonomic Classification & Prevalence of Arterial Lesions in Non-Human Primates*

			Number of Animals			% of Aorta Containing Lesions		
Order	Common Name	Scientific Name	Total	With Lesions	With Atheroma	Fatty Steaks	Fibrous Plaques	Other
Primates	Mongoose	Lemur mongoz	1	1			4	
	Ring-tailed lemur	Lemur catta	2					
	Slow loris	Nycticebus coucang	1					
	Senegal bushbaby	Galago senegalensis	2					
	Douroucouli	Aotus trivirgatus	7	1		tr		
	Red uakari	Cacajao rubicundus	4	2		tr, tr		
	Red howler	Alouatta seniculus	7	5		tr, tr, tr, 1, 2		
	Brown capuchin	Cebus apella	4	1			tr	
	Squirrel monkey	Saimiri sciureus	14	13	1	tr, tr, tr, tr, tr, 3, 3 10, 15, 40, 6, 7, 15	tr, 3, 3	
	Spider monkey	Ateles geoffroyi	1					
	Golden lion marmoset	Leontideus rosalia	1	1		1		
	Cotton-top tamarin	Leontocebus oedipus	2					
	Geoffroy's tamarin	Leontocebus geoffroyi	1					
	White tamarin	Leontocebus melanoleucus	1	1		tr		
	Pig-tailed macaque	Macaca nemestrina	3	2		tr, 5		
	Rhesus macaque	Macaca mulatta	2	2	1	tr, 5	1, 15	
	Black ape	Cynopithecus niger	1	1		tr		
	Anubis baboon	Papio doguera	26	16		tr,tr,tr,tr,tr,tr,tr, 1, 1, 1, 1, 2, 2, 10, 10,	tr, tr, tr, tr, 2, 2, 2	
	Grass monkey	Cercopithecus aethiops	1					
	Mandrill	Mandrillus sphinx	1	1		tr		
	Moustached monkey	Cercopithecus cephus	1	1		3	2	
	De Brazza's monkey	Cercopithecus neglectus	1					
	White-nosed guenon	Cercopithecus nictitans	1	1		tr	2	
	Talapoin monkey	Cercopithecus talapoin	2	1		tr		
	Patas monkey	Erythrocebus patas	2	2		tr, tr		
	Black & White colobus	Colobus polykomos	3	2		tr, 4	tr, 2	
	Red colobus	Colobus badius	1	1		tr		
	White-handed gibbon	Hylobates lar	5	2		tr, tr		
	Siamang	Hylobates syndactylus	1	1		tr		
	Chimpanzee	Pan troglodytes	2	2		15, 20		
	Orang-utan	Pongo pygmaeus	1	1		tr		
	Lowland gorilla	Gorilla gorilla	2	2		tr, 15		
		TOTALS	104	63	2	60	19	0
		%		61%	2%	58%	18%	

tr = Trace

mammals, 2 (2%) of 104 non-human primates, none of 54 carvivores, none of 12 pinnipeds, 17 (24%) of 72 birds, and 4 (10%) of 42 exotic mammals (Table I). A plaque was called atherosclerotic when focal calcification, necrosis, accumulations of extracellular lipid and/or deposits of doubly refractive crystals were present in the central portion of the lesion. The percentage of the surface of the aortas involved by atherosclerotic plaques could not be readily estimated, since the diagnosis of atherosclerosis was usually made only after microscopic inspection. The individuals with atherosclerotic lesions may be found in Tables III-VII. Atherosclerotic plaques were usually located in the abdominal segments of the aortas. The internal elastic lamella was often fragmented, but was sometimes intact, even beneath large atherosclerotic plaques. (Figures 5 and 6) No ulcerated atheromatous plaques were noted, although one lesion in the abdominal aorta of an elderly male rhesus monkey *(Macaca mulatta)* contained a central crater-like depression suggesting previous ulceration, and similar crater-like depressions were seen in plaques of several of the birds. One mural thrombus was found on microscopic examination loosely attached to a large fibrous plaque in the abdominal aorta of an adult female ostrich *(Struthio camelus)* (Figure 7). This bird died following surgical correction of a prolapsed rectum, so that sepsis may have been responsible for the thrombus. This was the only mural thrombus found in the entire study.

Medial Lesions: Various types of medial lesions were found in several species (Tables III-VII). Multiple saccular aneurysms were present in the thoracic aortas of two elderly black bears *(Ursus americanus)*. The mouths of these aneurysms measured from 5 to 25 mm. in diameter (Figure 8). The aneurysms were ap-



FIGURE 1.—(Z 512) Aardvark (Orycteropus axer). Adult female. Thoracic aorta showing numerous fatty streaks. Sudan IV stain.

parently due to foci of medionecrosis. No acutely necrotic smooth muscle cells were observed, but these cells were absent in large areas of the involved media, leaving sheets of hyalinized tissue containing sparse elongated nuclei. Elastic fibers at the margins of the aneurysms were fragmented, closely aligned, and often clumped and coiled in tangled masses (Figure 9). The intima overlying the aneurysms was greatly thickened and hyalinized, but no mural thrombi were present.

Somewhat different lesions were found in the thoracic aortas of an adult male tamandua *(Tamandua tetradactyla)* and an adult male Patagonian cavy *(Dolichotis patagonium)*. These lesions were also associated with multiple saccular aneurysms. Histologically, the lesions consisted of focal areas of degeneration and calcification of the smooth muscle elements of the inner media. The elastic fibers remained unaltered within these areas of calcification, except for the most central portions, which were completely replaced by granulation-like tissue (Figure 10).

Medial lesions of a different character were present in several species of hoofed mammals. These lesions usually occurred in the lower thoracic aortas, producing a gross picture of narrow longitudinal bands of transverse ridges. In one elderly female wapiti *(Cervus canadensis)*, the proximal inferior mesenteric and celiac arteries were transformed into washboard-like structures by this process (Figure 11). Microscopically, the elastic fibers seemed to be affected first, appearing swollen and containing stippled calcium deposits. Later, the entire inner media was replaced by areas of hyalinization with focal calcification. Contraction of the involved tissue apparently produced the transverse ridges that were visible to the naked eye.

The etiology of the three types of medial lesions described above was not obvious. Parasitic

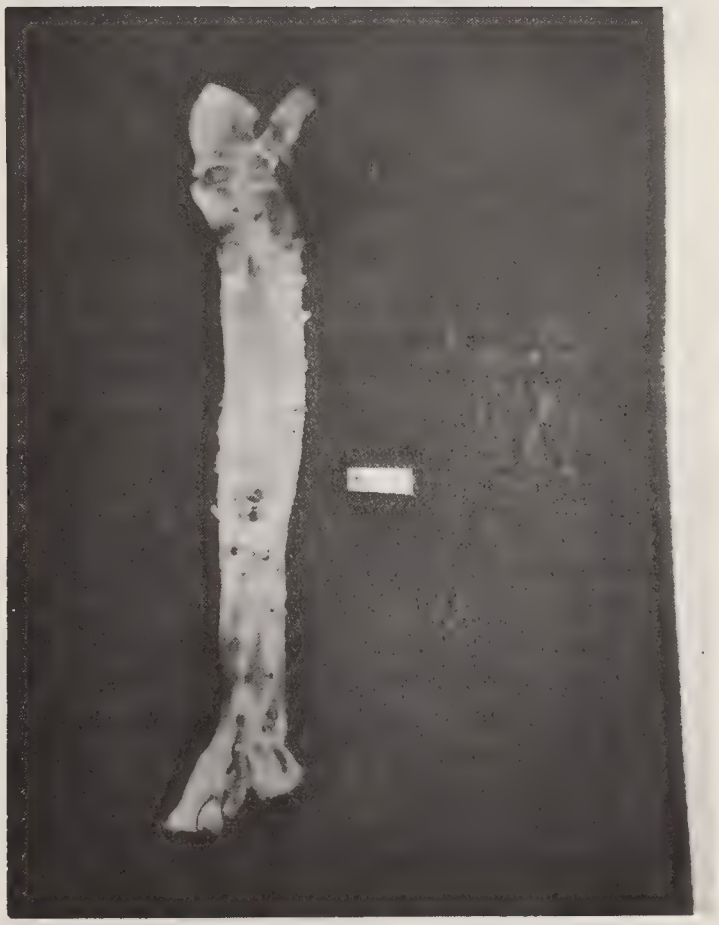


FIGURE 2.—(Z 497) Chimpanzee (Pan troglodytes). Nine-year old female. Entire aorta showing 15% fatty streaks. Sudan IV stain.

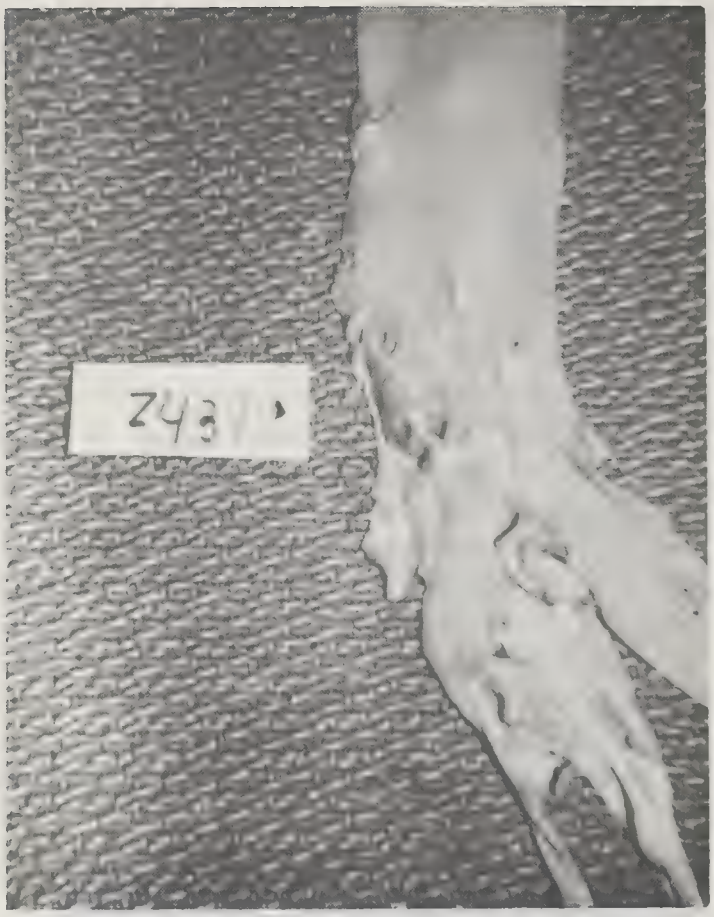


FIGURE 3.—(Z 431) Markhor goat (Capra falconeri). Young adult female. Abdominal aorta showing several fibrous plaques near the trifurcation. Sudan IV stain.

migration may produce aneurysms in the thoracic aortas of various animals. Although we did not find evidence for parasitism in these cases, we could not absolutely rule out this possibility. Parasitic lesions were present in the aortas of several animals, primarily carnivores (Tables III-VII).

Occasional medial lesions were found in birds, but nearly all of these appeared to be related to adjacent intimal disease. The medial lesions consisted of focal calcification, chondroid metaplasia, or accumulations of foam cells and/or doubly refractile crystals near the medio-intimal junction.

Coronary Artery Lesions

The epicardial coronary arteries from 84 mammals and birds were removed, stained with Sudan IV and graded for arteriosclerotic involvement. Fatty streaks and/or fibrous plaques were found in 12 (32%) of 38 hoofed mammals, 5 (29%) of 17 non-human primates, 2 (20%) of 10 carnivores, none of 8 pinnipeds, 4 (57%) of 7 birds, and 3 (60%) of 5 exotic mammals (Table II). The majority of these lesions were small, involving 5% or less of the intimal surface. In one white-tailed deer *(Odocoileus virginianus)* and one sitatunga *(Tragelaphus spekei)*, fibrous plaques covered 10% and 15% of the intimal surface, respectively (Table VIII). None of the lesions produced significant stenosis and no large myocardial scars or infarcts were found.

Microscopically, the fatty streaks consisted of slight intimal thickenings which contained Oil Red O positive droplets. The lipid was usually located within smooth muscle cells. In several non-human primates, birds and exotic mammals, typical foam cells were seen. The fibrous plaques were composed of longitudinally oriented smooth muscle cells and elastic fibers, and



FIGURE 4.—(Z 464) Ostrich (Struthio camelus). Young adult male. Abdominal aorta showing several fibrous plaques. Sudan IV stain.

TABLE V.—*Taxonomic Classification & Prevalence of Arterial Lesions in Carnivores and Pinnipeds*

Order	Common Name	Scientific Name	Number of Animals			% of Aorta Containing Lesions		
			Total	With Lesions	With Atheroma	Fatty Steaks	Fibrous Plaques	Other
Carnivora	Coyote	Canis latrans	5	2			tr, 1	*
	Weimaraner	Canis familiaris	2	2			tr, 1	
	Spotted hyena	Crocuta crocuta	1	1		2	2	
	Cacomistle	Bassariscus astutus	3	2		tr	2	*
	Raccoon	Procyon lotor	1	1			tr	
	African civet	Viverra civetta	1	1		4		
	Meerkat	Suricata suricatta	1					
	American badger	Taxidea taxus	1					
	Fisher	Martes Pennanti	1					
	Smooth otter	Lutra perspicillata	3				tr, tr	
	American black bear	Ursus Americanus	5	5		tr, tr, tr	5, 15, 20, 35	(+), (+)
	Asiatic black bear	Selenarctus thibetanus	1	1			tr	
	Sloth bear	Melursus ursinus	1	1		5	1	
	Mountain lion	Felis concolor	3					
	Tiger	Panthera tigris	4					
	African lion	Panthera leo	7					
	Black leopard	Panthera pardus	2					
	Cheetah	Acinonyx jubatus	1					
	Serval	Felis Serval	1					
	Bobcat	Felis rufa	1					
	Fishing cat	Felis viverrina	1					
	Margay	Felis wiedi	1					
	Leopard cat	Felis bengalensis	1					
	Golden cat	Felis temmincki	4	2			tr, 3	*
	Spotted cat	Felis rubiginosa	1	1			15	*
	Snow Leopard	Panthera uncia	1				tr	
		TOTALS	54	19	0	7	19	6
		%		35%		13%	35%	11%
Pinnipedia	Sealion	Zalophus californianus	7	7			tr, tr, 2, 5, 10, 10, 11	
	Harbor seal	Phoca vitulina	5	4			tr, 1, 2, 5	
		TOTALS	12	11	0	0	11	0
		%		92%			92%	

* = Parasitic lesions
\+ = Medial Degeneration and/or calcification; (+) = with aneurysms
tr = Trace

frequently contained lipid droplets. In one Nubian ibex *(Capra ibex)*, the lipid droplets were extremely numerous, yet no foam cells were present (Figure 12). Although none of the lesions were classified as atherosclerotic, fibrous plaques in two animals, one white-tailed deer *(Odocoileus virginianus)* and one stork *(Jabiru mycteria)*, contained central, hypocellular, lipid-rich zones which suggested early atheromatous transformation (Figure 13).

The coronary arteries in medium sized and small hearts were examined in 54 non-human primates using a modification of the method of Clarkson.[23] In medium-sized hearts blocks were removed from the main left coronary artery, the proximal portions of the left anterior descending and circumflex branches of the left coronary artery, and the proximal portion of the right coronary artery. Three frozen step sections from half of each block were stained with Oil Red O, and the remainder of the block was embedded in paraffin for a single H & E section. In the small hearts, one or two coronal blocks including both ventricles was removed and processed in the same manner. All of the arteries greater than 30 microns in diameter in the sections described were then counted, and the total was divided into the number of arteries with intimal disease to derive a score for each heart. Lesions consisting of minor intimal thickenings which contained Oil Red O positive material were found in 10 (19%) of the 54 hearts. One to 6.5% of the arteries were involved in the majority of the hearts with lesions. In one male bush baby *(Galago senegalensis)*, 11% of the small arteries had lesions. Significant arterial stenosis was not found in any of the hearts.[17]

The coronary arteries in medium sized and small hearts of all of the other species of mam-

TABLE VI.—*Taxonomic Classification and Prevalence of Arterial Lesions in Birds*

Order	Common Name	Scientific Name	Number of Animals			% of Aorta Containing Lesions		
			Total	With Lesions	with Atheroma	Fatty Steaks	Fibrous Plaques	Other
Sphenisciformes	Humboldt Penguin	Spheniscus humboldti	7	6	2	4, 4, tr, tr, tr, tr	4, tr, tr, 5, tr	*
Pelecaniformes	Pelican	Pelecanus occidentalis	2	2	1	tr, tr	tr, 2	
	Blue-footed boobie	Sula variegata	1	1		tr	tr	
Casuariiformes	Emu	Dromiceus novahollandie	1	1		12	3	
Struthioniformes	Ostrich	Struthio camelus	3	3	2	65, 60, 3	4, 3, 1	+, +
Rheiformes	Rhea	Rhea americana	3	3		tr, 5, 20	3, 5, 15	
Ciconiiformes	White stork	Ciconia ciconia	2	2		tr, 1	tr, tr	
	Jabiru stork	Jabiru mycteria	1	1	1	8	2	
	Greater marabou	Leptoptilos crumeniferus	1	1	1	3	5	
	Lesser marabou	Leptoptilos javanicus	1	1		tr	tr	
	Flamingo	Phoenicopterus ruber	1					
	Louisiana heron	Hydranassa tricolor	1	1	1	3	3	
	Goliath heron	Ardea goliath	2	2	2	15, tr	1, 15	
Anseriformes	Common duck		10	6		tr, 50, 3, 1, 1, 2	2	
	Common goose		3	3		1, tr, 3		
	Canadian goose	Branta canadensis	1	1			4	
	Red-breasted goose	Branta ruficollis	1	1		8		
	Ashey-headed goose	Chloephaga policephala	1	1		tr	tr	
	Versicolor teal	Anas versicolori	1	1		tr		
	Chinese top-knot goose	Anser cygnoides	2	2	1	3, tr	tr	
	Black swan	Chenopis atrata	1	1		tr		
	Black-necked swan	Cygnus melancoriphus	1	1		tr		
Falconiformes	Bald eagle	Haliaetus leucocephalus	1	1			tr	+
	Golden eagle	Aquila chrysaetos	1	1		5	5	
	Secretary bird	Saggitarius serpentarius	2	2		9, 5	tr, 2	
Galliformes	Domestic turkey		6	6	3	tr, tr, 15, 80, 10	tr, 2, 10, 5	
	Peafowl	Pavo cristatus	3	3	3	tr, 2, 10	15, 12, 10	+
Gruiformes	Sarus crane	Grus antigone	1	1		1	1	
	Crowned crane	Balearica pavonina	3	3		tr, 4, 4	tr	
	Wattled crane	Bugeranus carunculatus	1	1		1	tr	
Psittaciformes	Scarlet macaw	Ara macao	1	1		2		
	Yellow-headed parrot	Amazona ochrocephala	3	2		1, tr		
Coraciiformes	Ground Hornbill	Bucorvus abyssinicus	1	1		tr	tr	
Strigiformes	Snowy owl	Nyctea scandiaca	1	1		1		
Piciformes	Toucan	Ramphastos ambiguus	1	1		tr		+
		TOTALS	72	65	17	62	42	6
		%		90%	24%	86%	58%	8%

* = Fungal lesion
+ = Medial degeneration and/or calcification
tr = Trace

mals and birds included in the study were not systematically examined.

Cerebral Artery Lesions

The arteries of the Circle of Willis were examined systematically in the brains of 80 nonhuman primates. In the large brains, the circle was removed and the vessels opened longitudinally. In the great majority of brains which were too small to be examined by this method, the arteries were inspected unopened using a dissecting microscope or a hand lens. It is possible that very small intimal thickenings were overlooked by the latter method. However, it is doubtful that any lesion of significant size escaped detection, since most of these arteries were virtually transparent. Lesions were found in 2 of the 80 animals, a 3-year old male and a 9-year old female chimpanzee *(Pan troglodytes)*. Lipid rich plaques were present at the origin of the basilar and intracranial internal carotid arteries. These plaques contained many foam cells and occupied up to 50% of the luminal diameter. A hypocellular, lipid rich area with focal calcification characteristic of atherosclerosis was seen in the center of a lesion in the intracranial internal carotid artery of the older animal (Figure 14). There was no evidence of infarction of the brain in either animal.

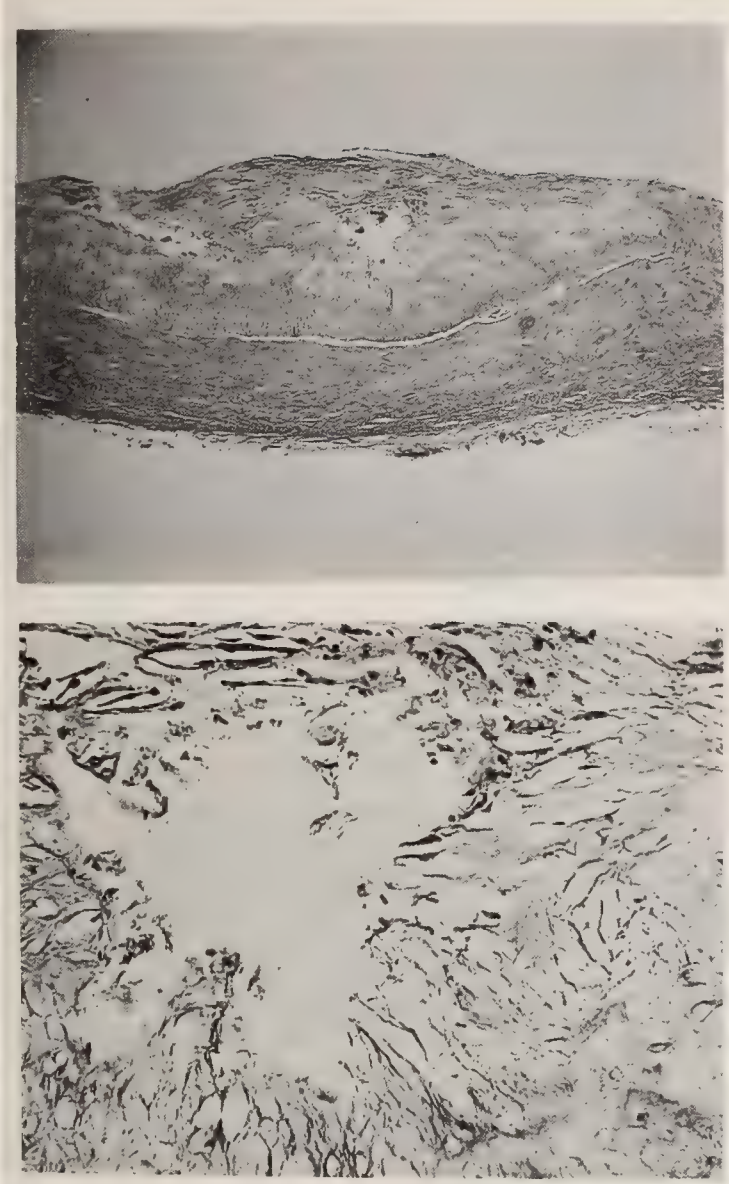


FIGURE 5.—(Z 316) Peacock (Pavo cristatus). Adult male.

A. Atheromatous plaque showing central necrosis and accumulation of lipids. H & E x 25.

B. Higher magnification showing central necrosis, cleft-like spaces and foam cells. H & E x 160. (Reproduced with permission from Exp. Molec. Path., Academic Press, New York).

The cerebral arteries were not systematically studied in the brains of the other species of mammals and birds.

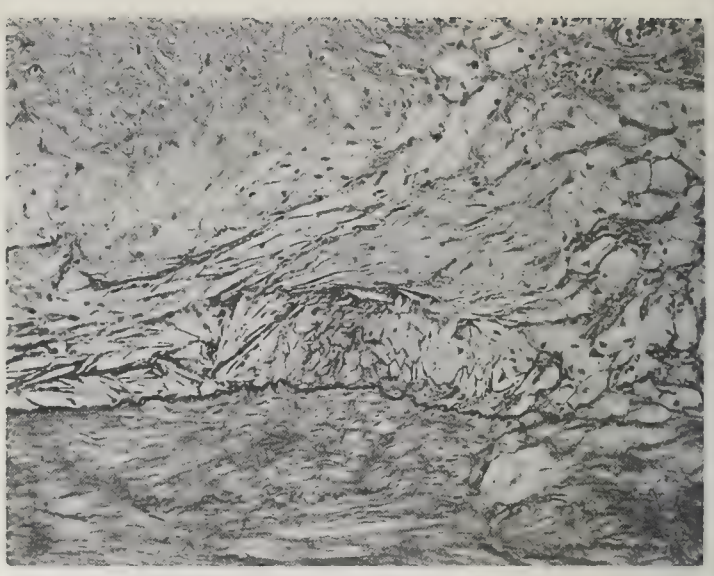
DISCUSSION

The findings in the aortas of the various species of captive mammals and birds are interesting for several reasons. As more and more surveys of this type are reported, it becomes clear that fatty streaks, fibrous plaques and atherosclerotic plaques occur in a large number of diverse species. In our material, and also that of Vastesaeger[9] and Finlayson,[10] true atherosclerotic plaques with areas of central necrosis and lipid and/or cholesterol accumulations, were seen much more commonly in birds than in any of the mammals or reptiles except for man. This may be due to the fact that birds have higher blood pressures[24] than mammals, and also higher serum cholesterol levels,[9,10] although admittedly, information on both of these parameters is limited. It does seem certain that being a bird is more important than the type of diet consumed, since fish eating birds have far more atherosclerosis than fish eating mammals, and carnivorous birds have far more atherosclerosis than carnivorous mammals. Furthermore, there does not appear to be any significant difference in the prevalence and severity of atherosclerosis among the birds themselves, whether the habitual diet is meat, fish, grain or insects.

The above statements are not intended to say that fish eating and carnivorous mammals have no arterial lesions. Fibrous plaques were common in these species, especially the harbor seals *(Phoco vitulina)* and the sealions *(Zalophus californianus)*, three of whom had 10% of their aortas involved by these lesions. It was interesting that these fibrous plaques, which resembled early human proliferative lesions in morphology and distribution, contained no Sudan IV or Oil Red O positive material. Moreover, the aortas of seals and sealions were completely devoid of fatty streaks. In view of the current theories of atherogenesis, one is tempted to conclude that this lack of arterial lipid is due to the favorable ratio of unsaturated to saturated fats in the diet. While this may be true, it will be of little consolation to those who feel that proliferation of smooth muscle cells in the intima in atherosclerosis is due to the accumulation of lipid within these cells. Several of the fibrous plaques in sealions had reached a thickness equal to the aortic media without the aid of stainable lipid (Figure 15).

Although it is risky to extrapolate from disease of animals to those of man, the hoofed mammals (Orders Artiodactyla and Perissodactyla) were another group in which lipids ap-





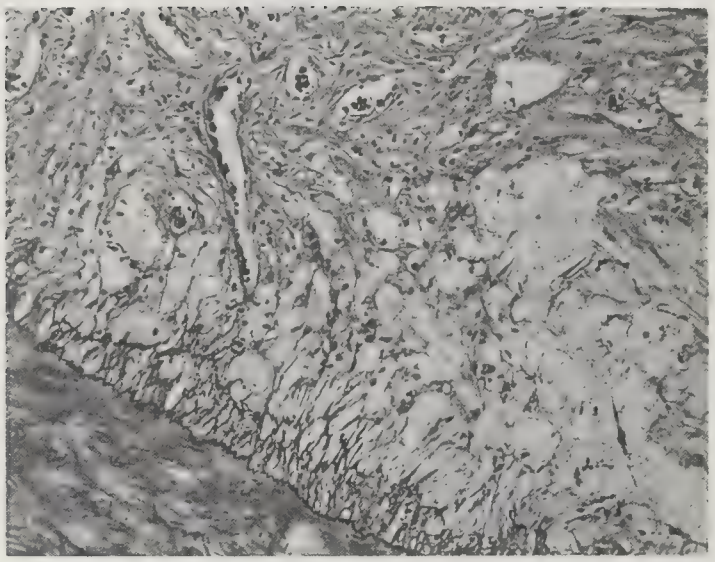


FIGURE 6.—(Z 481) Goliath heron (Ardea goliath). Adult male.

A. Large atheromatous plaque showing extensive accumulation of lipids and vascularization. Weigert's elastic stain x 25.

B. Higher magnification showing vascularization. Weigert's elastic stain x 160.

C. Higher magnification showing foam cells and cleft-like spaces. Weigert's elastic stain x 160. (Reproduced with permission from Exp. Molec. Path. Academic Press, New York).

peared to play very little part in the pathogenesis of the elevated arterial lesions observed. Fatty streaks were reasonably common in hoofed mammals, especially young ones, and were seen primarily in the thoracic segments of the aortas. Fibrous plaques were common in adult hoofed mammals, but were seen almost exclusively in the abdominal segments of the aorta. Therefore, it would appear that fatty streaks did not evolve into fibrous plaques.

Lipid was found in some of the larger fibrous plaques in hoofed mammals, but in these instances it was distributed throughout the lesion and sometimes into the adjacent media, or more frequently, was concentrated in the peripheral portions of the plaque. Foam cells were almost never seen, nor were central accumulations of lipid or areas or central plaque necrosis. On the other hand, in birds and a few non-human primates, areas of central necrosis were not uncommon, and accumulations of extracellular lipid were often seen in these areas. The ques-

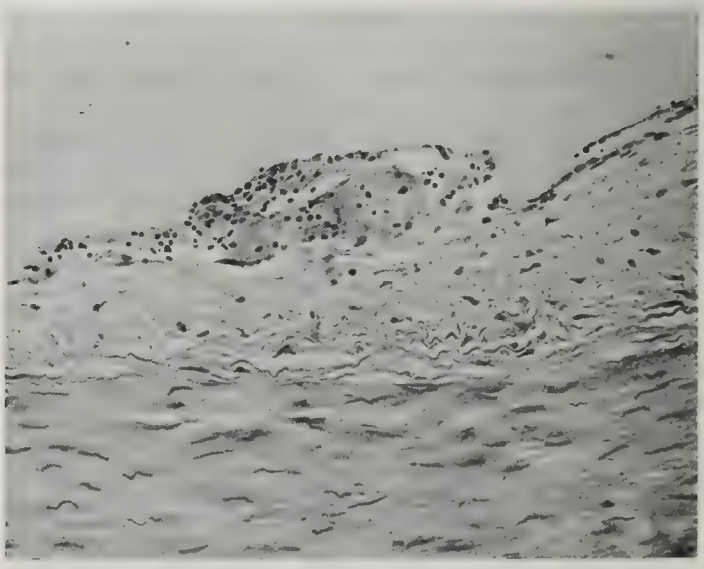


FIGURE 7.—(Z 405). Ostrich (Struthio camelus). Adult female. Small mural thrombus loosely attached to the surface of a fibrous plaque. H & E x 160.

TABLE VII.—*Taxonomic Classification and Prevalence of Arterial Lesions in Exotic Mammals*

Order	Common Name	Scientific Name	Number of Animals			% of Aorta Containing Lesions		
			Total	With Lesions	With Atheroma	Fatty Steaks	Fibrous Plaques	Other
Insectivora	Brandt's hedgehog	Paraechinus hypomelas	1	1	1	1		
	European hedgehog	Erinaceus europaeus	1					
Edentata	Nine-banded armadillo	Dasypus novemcinctus	3	1		tr		
	Great anteater	Myrmecophaga tridactyla	2	2	1	tr, 3		
	Tamandua	Tamandua tetradactyla	2	1	1	tr		+
	Two-toed sloth	Choloepus didactylus	2	2		tr, 1	tr	
Rodentia	Agouti	Dasyprocta aguti	1					
	Squirrel	Citellus sp.	3					
	Cloud rat	Phloeomys pallidus	1					
	American porcupine	Erethizon dorsatum	3					
	Tree porcupine	Coendou prehensilis	3					
	Capybara	Hydrochoerus hydrochaeris	5	4		tr, tr	tr, tr, tr, 1	
	Patagonian cavy	Dolichotis patagonum	2	2			tr	(+)
Marsupialia	Kangaroo	Macropus kanguru	1					
		Macropus robustus	7	1				+
Hyracoidea	Rock hyrax	Procavia capensis	3					
Tubulidentata	Aardvark	Orycteropus afer	2	2	1	2, 10	1, 1	
		TOTALS	42	16	4	11	8	3
		%		38%	10%	26%	19%	7%

+ = Medial degeneration and/or calcification; (+) = with aneurysms
tr = Trace

tion of whether or not lipid accumulation was responsible for the necrosis or vice versa could not be resolved. However, in the atherosclerotic plaques in giant anteaters *(Myrmecophaga tridactyla)* and aardvarks *(Orycteropus axer)*, central necrosis appeared to precede lipid accumulation.

The most striking feature in this survey of arteriosclerosis in mammals and birds was the lack of mural thrombi. Even in the individuals with multiple saccular aneurysms of the aorta, no mural thrombi were found. This is in marked contrast to the human situation, in which an aneurysm without a mural thrombus is a rarity. The paucity of mural thrombi in mammals and birds probably accounts for the fact that no ischemic lesions were seen in the present study, even in the presence of some rather large atherosclerotic plaques.

OTHER CARDIOVASCULAR DISEASES

Pre-Eclampsia

According to a recent review by Craig,[25] and a monograph by Douglas,[26] a spontaneous or experimental animal model of human pre-eclampsia has not yet been identified. We were therefore excited by the finding of glomerular capillary endothelial swelling, the pathognomonic renal lesion of pre-eclampsia in humans, in a 9-year old pregnant chimpanzee. The animel was a primigravida, and died during anesthesia in the eighth month of gestation. The anesthetic was given in order to treat ringworm lesions of the scalp which had been bleeding excessively for several days due to constant scratching. The animal had been somewhat lethargic and the ankles had appeared swollen for 2 to 3 weeks prior to death. At autopsy, the hematocrit value was 17 vol.%, which was consistent with the observed blood loss. The retroperitoneal and mediastinal soft tissues were edematous, and the kidneys appeared enlarged and pale. Microscopically the glomeruli were enlarged and bloodless. The walls of nearly all of the glomerular capillary loops showed marked thickening, with subsequent luminal narrowing. P.A.S. stains revealed that the capillary basement membranes were normal, and that the thickening was almost entirely due to vacuolic swelling of the cytoplasm of the endothelial cells (Figure 16). Hyaline droplets were present in the cytoplasm of a few of the endothelial and epithelial cells. There was no evidence of glomerulonephritis, pyelonephritis or any other renal disease. Examination of the remaining organs, including the placenta, disclosed no additional pertinent findings.[27]

TABLE VIII.—*Prevalence of Coronary Artery Lesions in Large Mammals and Birds*

Order	Common Name	Scientific Name	No. of Animals		% of Artery Containing Lesions			
					Right Coronary		Left Coronary	
			Total	With Lesions	Fatty Steaks	Fibrous Plaques	Fatty Steaks	Fibrous Plaques
Casuariiformes	Emu	Dromiceus novahollandie	1					
Struthioniformes	Ostrich	Struthio camelus	3	3	tr, tr	tr	tr, 1, 1	tr
Rheiformes	Rhea	Rhea americana	1					
Ciconiiformes	Jabiru stork	Jabiru mycteria	1	1	tr	tr		
Falconiformes	Secretary bird	Saggitarius serpentarius	1					
Pinnipedia	California sealion	Zalophus californianus	4					
	Harbor seal	Phoca vitulina	4					
Rodentia	Capybara	Hydrochoerus hydrochaeris	2					
Edentata	Giant anteater	Myrmecophaga tridactyla	1	1	tr	1	tr	tr
Tubulidentata	Aardvark	Orycteropus afer	2	2	1	1	1	
Carnivora	American black bear	Ursus americanus	5					
	Sloth bear	Melursus ursinus	1	1				2
	Weimaraner	Canis familiaris	1					
	Coyote	Canis latrans	1					
	Spotted hyena	Crocuta crocuta	1	1				7
	Snow leopard	Panthera uncia	1					
Primates	Chimpanzee	Pan troglodytes	2	2	3		1, 1	
	Lowland gorilla	Gorilla gorilla	1	1			tr	1
	Mandrill	Mandrillus sphinx	1					
	Baboon	Papio anubis	13	2				1, 5
Artiodactyla	Axis deer	Axis axis	1					
	Wapiti	Cervus canadensis	3	1				tr
	Mule deer	Odocoileus hemionus	2					
	Whitetail deer	Odocoileus virginianus	1	1		4		5
	Blackbuck	Antilope cervicapra	2	1			tr	tr
	Sitatunga	Tragelaphus spekei	3	1		15		
	Gemsbok	Oryx gazella	1	1				2
	Greater kudu	Tragelaphus strepsiceros	3	1				tr
	White-eared kob	Kobus kob	1					
	Sable antelope	Hippotragus niger	1					
	Nubian ibex	Capra ibex	2	1				tr
	Grant's gazelle	Gazella granti	6	2		1		tr, 1
	Defassa waterbuck	Kobus defassa	1					
	Angora goat	Capra hircus	2					
	Markhor goat	Capra falconeri	1					
	Persian gazelle	Gazella subgutturosa	1					
	Topi	Damaliscus korrigum	1	1				tr
	American bison	Bison bison	1					
	Siberian argali	Ovis ammon	1	1		1		1
	Cape Hartebeest	Alcelaphus buselaphus	1	1			1	1
	Africa guinea hog	Potamochoerus porcus	2					
	Dromedary camel	Camelus dromedarius	1					
		TOTALS	84	26	6	8	11	17

tr = trace

It seems peculiar that glomerular capillary endothelial swelling has not been previously reported in an animal, particularly since research on non-human primates has increased so markedly in the last decade. The reversible character of the lesion is undoubtedly partially responsible, along with the fact that good fixation and thin microscopic sections are necessary to adequately delineate the pathological changes. There appears to be no logical reason to assume that pre-eclampsia is confined to the human race. Percutaneous renal biopsies during the late stages of pregnancy in chimpanzees and other non-human primates might well uncover additional examples of this disease.

Essential Hypertension

Although hypertension can be induced experimentally by a variety of methods in several species, there is at present no spontaneous or experimental animal model for essential hypertension in humans. We were pleasantly surprised to discover cardiovascular changes suggesting essential hypertension in a chimpanzee. The animal was a 20-year old female who was

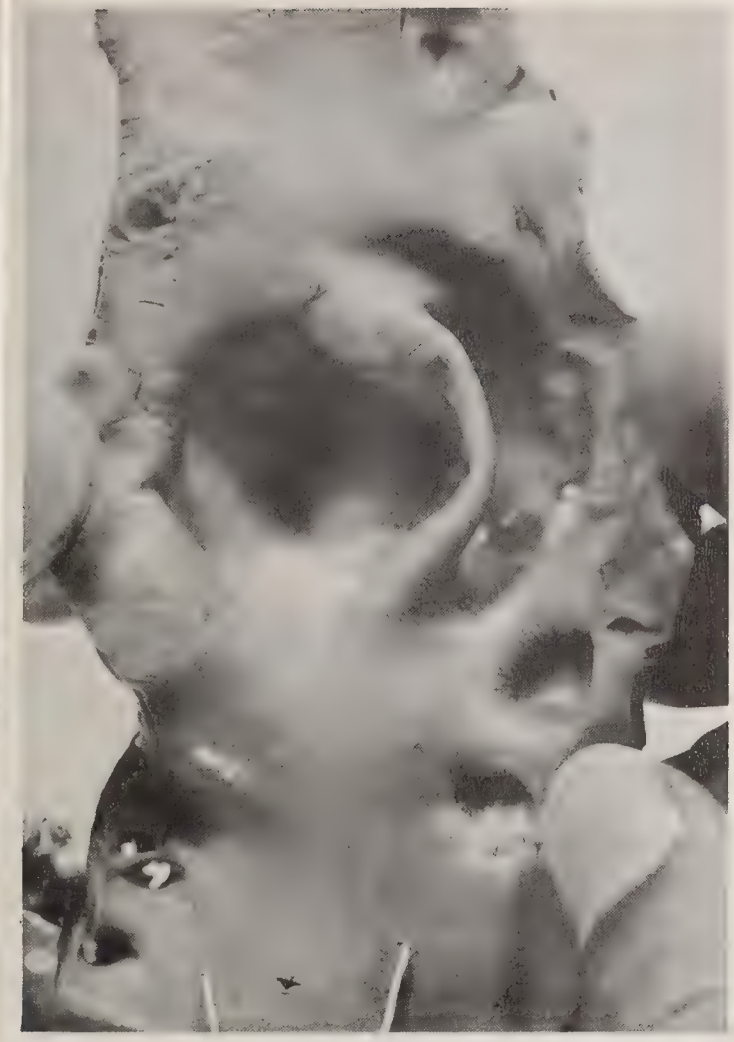


FIGURE 8.—(Z 178) Black bear (Ursus americanus). Elderly adult female. Thoracic aorta showing multiple saccular aneurysms. (Reproduced with permission from Amer. J. Path., Harper and Row, Hagerstown, Maryland).

found dead unexpectedly one morning. She had been in captivity for the last 14 years of her life, and had enjoyed apparent good health. Death was due to acute pulmonary edema, and the presence of hemosiderin laden macrophages in the alveolar spaces suggested chronic left ventricular failure. The left ventricle of the heart was tremendously hypertrophied, averaging 2.0 to 2.5 cm. in thickness. The right ventricle showed mild hypertrophy, measuring 0.3 to 0.4 cm. in thickness (Figure 17). There was no valvular abnormality or other obvious cause for the hypertrophy. The main, interlobar and arcuate renal arteries were normal. However, the interlobular renal arteries showed classical pseudoelastic hyperplastic sclerosis, and there



FIGURE 9.—(Z 141) Black bear (Ursus americanus). Elderly adult male. Section through the rim of small aneurysm showing loss of medial smooth muscle and elastic tissue. The overlying intima is thickened by hyaline-like tissue. Weigert's elastic stain x 25. (Reproduced with permission from Amer. J. Path., Harper and Row, Hagerstown, Maryland).

was focal, pronounced medial and intimal hypertrophy and hyperplasia in the preglomerular and slightly larger arterioles. Focal hyalinization of the afferent arterioles was also seen (Figure 18). These changes were exactly simi-



FIGURE 10.—(Z 485) Tamandua (Tamandua tetradactyla). Adult male. Section through a small aneurysm showing degeneration with expansion of smooth muscle cells with preservation of elastic fibers. Centrally, the entire inner media is replaced by granulation-like tissue. The overlying intima is thickened by hyaline-like tissue. Weigert's elastic stain x 25. (Reproduced with permission from Atherosclerosis, Elsevier, Amsterdam).



FIGURE 11.—(Z 251) Wapiti (Cervus canadensis). Elderly adult female. Inferior mesenteric artery showing multiple transverse ridges due to degeneration and hyalinization of the inner media. Sudan IV stain.

lar to those which have been described in the renal arteries and arterioles of humans with essential hypertension,[28] and were not present in sections from kidneys of 9 other chimpanzees, 4 of whom were over 35 years of age. No other abnormalities which could cause hypertension were found in the remaining organs.[29]

The blood pressure was not measured during life in the above described chimpanzee. The enormous difficulties involved in determining accurate, average blood pressures in non-human primates are probably responsible for the fact that no examples of hypertension have been uncovered.

DISCUSSION

The discovery of morphologic changes suggesting pre-eclampsia and essential hypertension in chimpanzees should stimulate investiga-



FIGURE 12.—(Z 488) Nubian ibex (Capra ibex). Adult female. Fibrous plaque from left coronary artery showing extensive lipid accumulation in the form of diffusely dispersed fine droplets. Oil Red O —hematoxylin x 25.

tors to look for these and other disease entities in living non-human primates. A living chimpanzee with pre-eclampsia or essential hypertension would be extremely valuable in biomedical research. For example, in a hypertensive animal, vascular resistance could be determined acutely and/or chronically by instrumentation

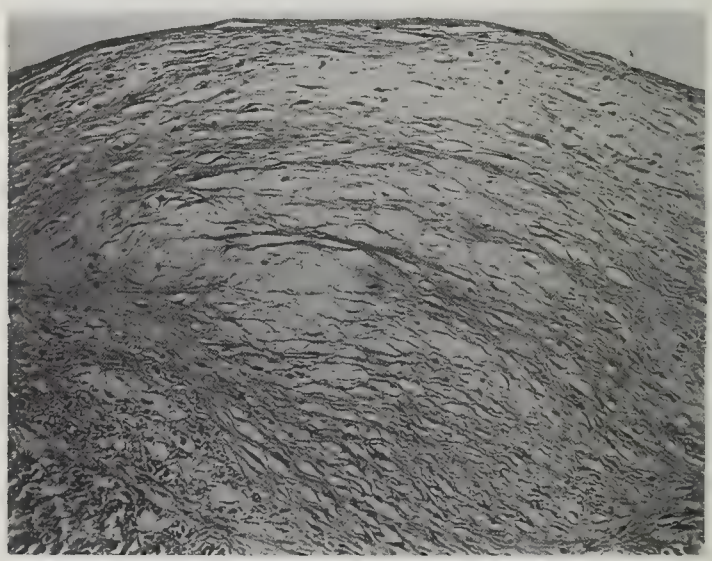


FIGURE 13.—(Z 400) Stork (Jabiru mycteria). Adult female. Fibrous plaque from right coronary artery showing marked expansion of cells in center of plaque by lipid accumulation. The media is just visible at the lower left corner. H & E x 160.

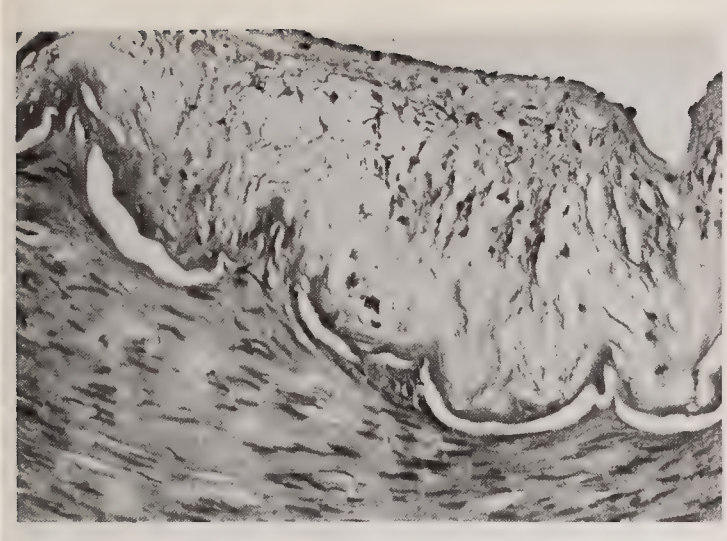


FIGURE 14.—(Z 497) Chimpanzee (Pan troglodytes). Nine-year old female. Hypocellular, lipid-rich plaque from the intracranial internal carotid artery. H & E 160. (Reproduced with permission from Exp. Molec. Path., Academic Press, New York).

in appropriate organs or vascular beds. The effects of various therapeutic agents on specific vascular beds could then be measured. In addition, portions of the central nervous system could be chronically stimulated to produce certain emotional states or behaviour patterns, with parallel measurements of vasomotor tone in appropriate vascular beds, and so on.

SUMMARY

The material presented was gathered during a five-year period from autopsies on animals dying in the Oklahoma City Zoo and the psychological research laboratory of Dr. W. B. Lemmon at the University of Oklahoma. Aortas and epicardial coronary arteries from large hearts were removed, flattened on cardboard, stained with Sudan IV and graded for intimal involvement using a modification of the method originally described by Holman. Contiguous microscopic sections of the various arterial lesions were stained with Oil Red O, H & E and Weigert's elastic stain. Typical atherosclerotic lesions were found in many of the birds, and smaller atherosclerotic lesions were found in a variety of other mammals, including anteaters, aardvarks and a Zebu cow. Atherosclerotic lesions were also present in non-human primates, particularly squirrel monkeys and chimpanzees. Chimpanzees were the only animals in which significant atherosclerotic lesions were present in the large coronary and cerebral arteries. None of these lesions were of clinical importance, however. Proliferative intimal lesions consisting primarily of smooth muscle cells were found in almost every species examined. In seals and sealions, these lesions were distributed in the aorta in a fashion similar to that seen in atherosclerosis in man. It was interesting that no lipid was present in the lesions of the seals and sealions, particularly since lipid





FIGURE 15.—A. (Z 446) Sea lion (Zalophus californianus). Young adult male. Small lipid-free intimal plaque composed of smooth muscle cells. H & E x 160.

B. (Z 415) Sea lion (Zalophus californianus). Young adult female. Large lipid-free musculoelastic intimal plaque. Weigert's elastic stain x 25. (Reproduced with permission from Amer. J. Path., Harper and Row, Hagerstown, Maryland).

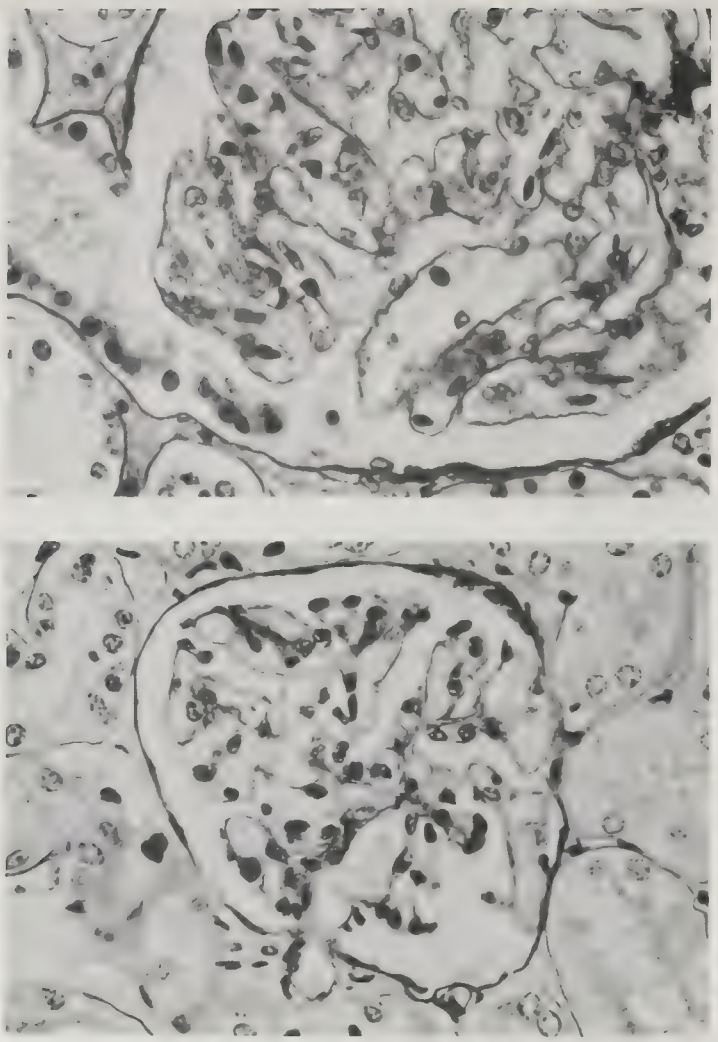


FIGURE 16—(Z 497) Chimpanzee (Pan troglodytes). Nine-year old female.

A. Glomerulus showing marked vacuolar and fibrillary thickening of capillary endothelial cells and overall enlargement. PAS x 400.

B. Normal chimpanzee glomerulus (courtesy Dr. H. L. Ratcliffe). PAS x 400. (Reproduced with permission from Amer. J. Obstet. and Gyn., C. V. Mosby, St. Louis).

insudation is thought to stimulate smooth muscle cell proliferation in the early stages of human atherosclerosis. It was also interesting that mural thrombi were almost never seen.

Degenerative medial lesions were found in hoofed mammals, anteaters, rodents and bears. Some of these lesions resembled the medionecrosis which occurs in humans, although none showed cystic changes. These medial lesions produced aneurysms in several animals.

In addition to the above findings, morphologic changes in two chimpanzees suggested a diagnosis of pre-eclampsia and essential hypertension, respectively. Pronounced glomerular capillary endothelial swelling was seen in a nine year old chimpanzee dying during the third trimester of her first pregnancy. In the second chimpanzee, a 20-year old female, the combination of left ventricular cardiac hypertrophy and proliferative sclerosis of the small renal arteries suggested long standing hypertension. Because of the absence of other changes known to produce hypertension in man and animals, this animal was thought to have had essential hypertension. The study of models such as these during life might yield considerable useful biomedical information.

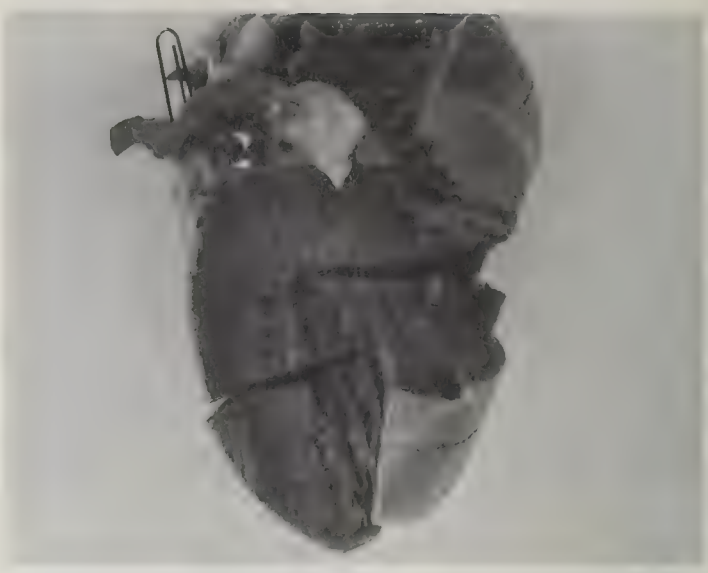
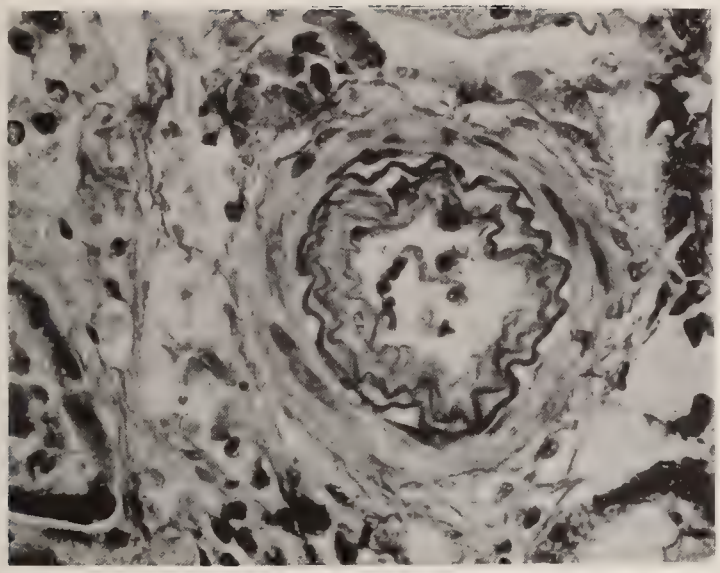
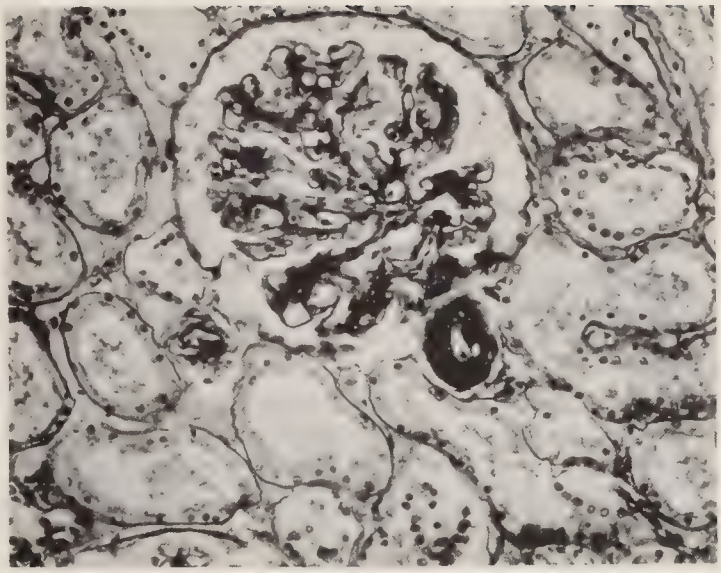


FIGURE 17.—("Maud") Chimpanzee (Pan troglodytes). Twenty-year old female. Photograph of the heart showing marked left ventricular hypertrophy. (Reproduced with permission from Exp. Molec. Path., Academic Press, New York).

ACKNOWLEDGMENTS

The efforts of Dr. Marshall E. Groover, whose imagination and persistence resulted in the funding and the initiation of the project are gratefully acknowledged. Dr. Groover was the principle investigator until his departure from the University of Oklahoma Health Center in 1965. Dr. Clarke Stout then assumed project responsibility. The generous financial assistance of the Oklahoma Zoological Society and the interest and cooperation of Dr. Warren Thomas and Dr. Philip Ogilvie and their respective





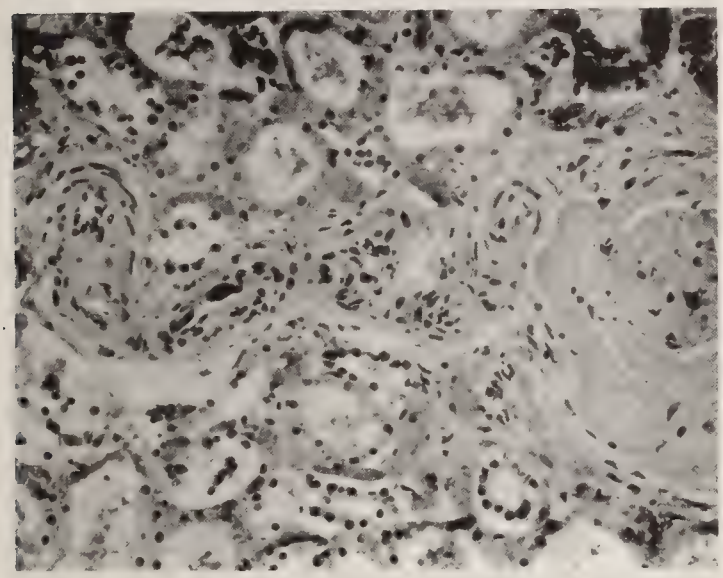


FIGURE 18.—("Maud") Chimpanzee (Pan troglodytes). Twenty-year old female.

A. Interlobular renal artery showing pseudoelastic hyperplastic sclerosis. Verhoeff van Geison stain x 400.

B. Marked intimal and medial proliferation in a renal afferent arteriole with ischemic atrophy of the supplied glomerulus. H & E x 160.

C. Hyalinization of renal afferent arteriole. The normal efferent arteriole is seen on the left. PAS x 160. (Reproduced with permission from Exp. Molec. Path., Academic Press, New York).

staffs at the Oklahoma City Zoo were also crucial to the success of the program. Mrs. Zelma Proctor is commended for the preparation of histologic sections of high quality.

REFERENCES

1. CLARKSON, T. B., PRICHARD, R. W., NETSKY, M. O., and LOFLAND, H. B. Atherosclerosis in pigeons. Its' spontaneous occurrence and resemblance to human atherosclerosis. Arch. Path. 68:143–147, 1959.
2. MIDDLETON, C. C., CLARKSON, T. B., LOFLAND, H. B., and PRITCHARD, R. W. Atherosclerosis in the squirrel monkey. Naturally occurring lesions of the aorta and coronary arteries. Arch. Path. 78:16–23, 1964.
3. MCGILL, H. C., JR., STRONG, J. P. HOLMAN, R. L., and WERTHESSEN, N. T. Arterial lesions in the Kenya baboon. Circ. Res. 8:670–679, 1960.
4. MORELAND, A. F., CLARKSON, T. B., and LOFLAND, H. B. Atherosclerosis in "Miniature" swine. I. Morphologic aspects. Arch. Path. 76:203–210, 1963.
5. MALINOW, M. R., and MARRUFFO, C. A. Aortic atherosclerosis in free-ranging howler monkeys. Nature, 206:948–949, 1965.
6. ROBERTS, J. C., and STRAUS, R., Eds. *Comparative Atherosclerosis.* Harper and Row, New York, 1965.
7. FOX, H. Arteriosclerosis in lower mammals and birds: Its relation to the disease in man. In: *Arteriosclerosis,* E. V. Cowdry, Ed., MacMillan, New York, 1933, p. 153–193.
8. RATCLIFFE, H. L., and CRONIN, M. T. I. Changing frequency of arteriosclerosis in mammals and birds at the Philadelphia Zoological Garden. Review of autopsy records. Circulation 18:41–52, 1958.
9. VASTESAEGER, M. M., and DELCOURT, R. The natural history of atherosclerosis. Circulation 26:841–855, 1962.
10. FINLAYSON, R. Spontaneous arterial disease in exotic animals. J. Zool. 147:239–343, 1965.
11. LINDSAY, S., and CHAIKOFF, I. L. Naturally occurring arteriosclerosis in animals: A comparison with experimentally induced lesions. In: *Atherosclerosis and Its Origin,* M. Sandler and G. H. Bourne, Eds., Academic Press, New York, 1963, p. 349–437.
12. DAHME, E. G. Atherosclerosis and arteriosclerosis in domestic animals. Ann. N. Y. Acad. Sci. 127:657–670, 1965.

13. Cornelius, C. E. Animal models—A neglected medical resource. New Eng. J. Med. 281:934–944, 1969.
14. Ratcliffe, H. L., Yerasimides, T. G., and Elliott, G. A. Changes in character and location of arterial lesions in mammals and birds in the Philadelphia Zoological Garden. Circulation 21:730–738, 1960.
15. Strong, J. P. Personal communication.
16. Holman, R. L., McGill, H. C., Jr., Strong, J. P., and Geer, J. C. Techniques for studying atherosclerotic lesions. Lab. Invest. 7:42–47, 1958.
17. Stout, C. Atherosclerosis in subhuman primates. In: *Proceedings of Symposium on the Use of Subhuman Primates in Drug Evaluation*, H. Vagtborg, Ed., Univ. Texas Press, Austin, Texas, 1968, p. 494–504.
18. Stout, C., and Bohorquez F. Aortic atherosclerosis in hoofed mammals, J. Atheroscler. Res. 9:73–80, 1969.
19. Stout, C., and Lemmon, W. B. Predominant coronary and cerebral atherosclerosis in captive nonhuman primates, Exp. Molec. Path. 10:312–322, 1969.
20. Stout, C. Atherosclerosis in exotic Carnivora and Pinnipedia, Amer. J. Path. 57:673–687, 1969.
21. Bohorquez, F., and Stout, C. Arteriosclerosis in exotic mammals. Atherosclerosis 16: 225–231, 1972.
22. Bohorquez, F., and Stout, C. Aortic atherosclerosis in exotic avians. Exp. Molec. Path. (currently in printing).
23. Clarkson, T. B., Prichard, R. W., Lofland, H. B., and Goodman, H. O. Interactions among dietary fat, protein and cholesterol in atherosclerosis—susceptible pigeons, Circ. Res. 11:400–404, 1962.
24. Sturkie, P. D. *Avian Physiology*, Cornell University Press, Ithaca, New York, 1965.
25. Craig, J. M. Models for obstetrical and gynecological disease, Fed. Proc. 28:206–210, 1969.
26. Douglas, B. H. *Experimental Approaches to Toxemia of Pregnancy*, Charles C. Thomas, Springfield, Illinois, 1971.
27. Stout, C., and Lemmon, W. B. Glomerular capillary endothelial swelling in a pregnant chimpanzee. Amer. J. Obstet. and Gyn. 105:212–215, 1969.
28. Stoddard, L. D., and Puchtler, H. Human renal vascular lesions and hypertension. In: *Pathology Annual*, S. C. Sommers, Ed., Appleton-Century-Crofts, New York, 1969, Vol. 4, p. 253–268.
29. Stout, C., and Lemmon, W. B. Hypertensive-like vascular lesions in a chimpanzee, Exp. Molec. Path. 14:151–157, 1971.

DISCUSSION

Benjamin T. Cole, University of South Carolina, Columbia: I may have missed this in your introduction, but were the 405 or more aortas taken from animals which simply died? In other words, you don't correlate the presence of the lesion with death or the age of the animal? Is there any correlation with your data between age?

The reason I bring this up is that there have been studies made in human aortas by someone at the Medical School at LSU where aortas were taken and a similar study done. His sample covered only decedents who had no history of cardiovascular disease. He found the interesting correlation that lesions were present in prepubertal humans. They seemed not to be so prolific during the period of active sexual life, (normally from puberty to menopause) but appeared again in aging persons. So my original two questions relate to that problem.

Dr. Stout: I didn't mention that, and I want to apologize, especially to the projectionist, for racing through the presentation. We did more or less consecutive autopsies for this five year period, so there was no obvious selection. In general, the lesions were positively correlated with advancing age, but not with sex. We could not correlate the lesions with any disease process simply because there were too many different species involved, and too many different causes of death. Also, in a number of cases, we could not determine the exact cause of death.

Dr. Cole: That answers my question. The interesting aspect of the work in human aortas was the possible endocrine factor in this normal event of clot formation which never becomes excessive enough to cause any clinical abnormality.

Wilbur P. McNulty, Oregon Primate Center, Beaverton, Oregon: Did the lesions correlate at all with length of time in captivity independent from age?

Dr. Stout: Our records were not as complete as those at the Philadelphia Zoo, so that I can't answer the question. We did try to make this determination in birds, but were unable to see any definite correlation between the length of time in captivity and the severity of arteriosclerosis.

Keith McCullagh, Cleveland Clinic: I would like to make a comment and a question. The comment regards Dr. Cole's question concerning age and spontaneous atherosclerosis. I did a study in East Africa with about the same

number of wild African elephants some years ago. We found a definite correlation between the age and the incidence of atherosclerosis. We also found a difference between two different populations of elephants. Both groups had an age-related change, but one population had a higher level than the other. So to conclude this is normal in wild animals is to also conclude that other factors are also involved.

The question I would like to ask Dr. Stout refers to other studies of zoological material, notably Dr. Finlayson's in London, where findings show the most severe form of atherosclerosis seems to be in wild animals, particularly the hoofed type of horse, rather than in the non-human primates, which are closest to man. Do your studies confirm this theory?

DR. STOUT: Our advanced atherosclerotic lesions were much more common in birds. There were lots of lesions in the hoofed animals, but most of them were uncomplicated fibrous plaques. Medial degenerative lesions were more extensive in hoofed mammals, and these lesions often had a secondary intimal component. Possibly this is what you are referring to.

ANN FREE, "Washington Evening Star": Could I ask a question, please? My name is Ann Free. I'm from the "Washington Evening Star." Could you tell us something about the exercise facilities—the type of caging available in the Oklahoma Zoo. Also, do you think the lack of muscle tone or built-up tension could have had any effect?

DR. STOUT: Well, Oklahoma is the wide open spaces, and the Zoo is about the same, being designed so that the animals have quite a bit of room in which to move about. However, for the reasons cited above, we were unable to correlate factors such as these with the severity of arteriosclerosis.

ALTERATIONS IN DNA SYNTHESIS AND OTHER METABOLIC PROCESSES IN AORTAS OF CHOLESTEROL-FED SWINE

W. A. Thomas, R. A. Florentin, S. C. Nam
J. M. Reiner and K. T. Lee*

Swine fed a high fat-cholesterol diet begin to develop gross atherosclerotic lesions in 1–2 months. The most prominent histologic features of the early gross lesions are excessive accumulation of lipid-laden smooth muscle cells (SMC), collagen, elastin and mucopolysaccharides in arterial intimas. Metabolic and cell kinetic studies of the gross lesions indicate increased rates of DNA and protein synthesis, oxygen consumption and cell division. Electron microscopy studies show changes in a majority of SMC that are consistent with increased activity; however a significant minority are degenerating or dead. Investigation of metabolic and cell kinetic changes that occur in arteries of high fat-cholesterol-fed swine prior to development of atherosclerotic lesions recognizable by either gross or light microscopy have been made. As early as 3 days on high fat-cholesterol diet increased rates of DNA synthesis, oxygen uptake and cell division can be demonstrated. By electron microscopy a small but significantly increased number of dead or degenerating cells can be seen. Studies of the cell cycle indicate that most arterial SMC are in the dividing population and that the increased cell division rate results from shortening of the postmitotic, pre-DNA synthesis period (G_1 and G_0). By 3 days on the high fat-cholesterol diet serum cholesterol levels will have risen from approximately 100 mg% to 150–200. However, no increase in concentration of cholesterol in the arterial wall has been demonstrated before at least 1–2 months. Also study by electron microscopy of SMC in mitosis at 3 days on diet shows no recognizable lipid droplets. Thus metabolic and cell kinetic changes in arterial SMC appear to antedate excessive accumulation of cholesterol. This apparent paradox has not yet been explained.

INTRODUCTION

We are investigating metabolic changes that take place in arteries of cholesterol-fed swine and other animals in the early stages of atherogenesis, including those that occur prior to the development of grossly visible atherosclerotic lesions. When young swine are fed atherogenic diets such as those shown in Table I, they begin to develop grossly visible atherosclerotic lesions in the arch and in the distal portion of the abdominal aorta, as well as in the coronaries and other arteries, in approximately one month.[1,2] These lesions increase in size and number with time. The rate of increase appears to depend on the degree of hypercholesterolemia produced by the diet. Table II shows serum cholesterol levels associated with the various diets of Table I in a typical experiment.

* Department of Pathology and Specialized Center of Research in Atherosclerosis, Albany Medical College, Albany, New York.

METHODS AND MATERIALS

Most of the data presented are from experiments using the mild diet which produces serum cholesterol levels similar to those of young adult North Americans. On the mild diet atherosclerotic lesions usually increase in number and size

TABLE I.—*Composition of diets consumed per day in grams*

Ingredients	"Stock" (PS)	"Mild" (PSC)	"Severe" (TSC)	"Moderate" (SC)
Casein	143	143	95	152
Sucrose	227	227	140	224
Butter	57	57	87	139
Peanut oil	57	57	87	139
Salt mix, Wesson	30	30	30	48
Vitamin mix	12.5	12.5	12.5	20
Choline Chloride	2.5	2.5	2.5	4
Cellulose	121	113	24.5	41
Cholesterol	0	8	14	22
Sodium cholate	0	0	7	11
Propylthiouracil	0	0	0.5	0
Total	650	650	500	800
Calories	2,506	2,506	2,506	4,006

TABLE II.—*Mean Serum cholesterol levels*

Initial	90 mg%
Final	
Stock diet group	141 mg%
Mild diet group	185 mg%
Severe diet group	1525 mg%
Moderate diet group	480 mg%

rather slowly as is the case in man. Even after a year on the mild diet the swine will have relatively few small lesions. With the severe diet lesions progress much more rapidly; and after a year on the diet there will be near occlusion of many coronary arteries. The rapidity with which the lesions progress can be increased even more if some form of physical injury to arteries is given in addition to the hypercholesterolemic diet. For example, if deep x-irradiation is given to the coronary arteries of hypercholesterolemic swine, even in doses that produce no significant morphologic changes by themselves, the rapidity of growth of lesions will be greatly increased. We have done this in conjunction with both the moderate and the severe diet; and occlusive lesions develop in 4 to 5 months with associated myocardial infarction and often "sudden death," which we have shown to be due to ventricular fibrillation in many instances.

The morphologic features of the atherosclerotic lesions as seen by light and electron microscopy are basically similar in swine fed the mild, moderate, or severe diet with or without x-irradiation. As in man, there is excessive accumulation of modified smooth muscle cells (SMC) in the intima and inner media.[3] Many of the SMC in the lesions are filled with lipid droplets, and these cells usually show variable degrees of degenerative changes. Some cells in the lesions are so packed with lipid and so degenerate as to make absolute identification virtually impossible. This has led to a minor controversy among morphologists as to whether they originate as SMC or as macrophages. The distinction is probably not important, since the modified SMC in lesions appear to carry out many functions other than contraction. For example, lesions usually contain a great deal of collagen, and yet recognizable fibroblasts are seldom if ever present. The SMC can apparently synthesize collagen, elastic tissue, and perhaps many other proteins.

In earlier studies of atherosclerotic lesions in swine fed only the mild diet we have shown an increased rate of deoxyribonucleic acid (DNA) synthesis resulting from an increased number of cells synthesizing DNA at any one time.[1,4] As might be expected, oxygen consumption is increased along with the increase in DNA synthesis.[5] We have developed methods for separating mitochondria from arterial tissue so that respiratory control can be studied *in vitro*.[6] Mitochondrial activity of aortic intima-media preparations from swine with substantial atherosclerotic lesions appear to be similar to those from controls.[7] Thus significantly increased uncoupling of oxidative phosphorylation, which one investigator has suggested occurs in arteries of atherosclerotic rabbits,[8] does not appear to be a feature of disturbed arterial metabolism in atherosclerotic swine. We have also carried out studies of protein synthesis in arteries with early lesions and the rate appears to be considerably increased.[9]

The conclusion to draw from the above studies of metabolism in arteries of swine with early atherosclerotic lesions is that overall metabolic activity, including DNA and protein synthesis, is enhanced. This is consistent with the great increase in SMC, collagen, and elastic tissue associated with the development of lesions. The increased oxygen uptake seems to indicate that the lesions do not result from oxygen deprivation as has been suggested for human lesions by some investigators in the past.

Along with the overall rise in metabolic activity is an apparent increase in the rate of degeneration and death of cells in the lesions.[10,11] This conclusion has to be based only on light and electron microscopy observations, since biochemical methods for detecting and quantitating the degenerative aspects have not been perfected. Current studies on activities of lysosomal enzymes in arteries by several laboratories, including our own, may provide new information on the degenerative factors in atherosclerosis.

The significance of greater metabolic activity in arteries with early atherosclerotic lesions is difficult to assess. Clearly, when cells proliferate excessively, there must be an accompanying increase in energy production, and this requires a greater consumption of either glucose or oxygen or both. This tells us nothing about cause and effect. We decided that study of metabolic changes that occurred in the arteries of cholesterol-fed swine prior to the development of gross lesions might provide more readily inter-

pretable information on atherogenesis than studies of lesions. Was increased arterial metabolic activity a feature long before the development of gross lesions, or was it preceded by a phase in which one or more metabolic functions were depressed?

We decided to study metabolic features in the period beginning with the first day on diet on a continuing basis until the development of gross lesions, approximately one month later. In the first study in this series[1] we found that swine fed the mild diet had increased DNA synthesis in their arteries in the first 10 days on diet. In a subsequent study we demonstrated a higher rate of entry of arterial SMC into mitoses as early as 3 days on diet,[4] reflecting increased numbers synthesizing DNA. Oxygen uptake in this early period was also elevated.[5] Other metabolic features have not as yet been intensively studied; but it seems likely that at least some others will show a similar pattern. The mitosis study is illustrated in Table III.

Thus increased activity, at least in DNA synthesis, and oxygen consumption, begins shortly after the diet is started, long before development of gross atherosclerotic lesions and even before a measurable increase in cholesterol concentration in the arterial wall has been demonstrated. No depression of any metabolic function has as yet been demonstrated in this early period on diet. However, by electron microscopy, counts have been made of arterial SMC that appear to be dead or dying in swine fed the mild diet for 3 days; and the numbers are somewhat greater than in controls.[11]

The increase in number of SMC synthesizing DNA so early after beginning the cholesterol diet is reminiscent of the increase in DNA synthesis in salivary glands shortly after a parenteral injection of isoproterenol.[12] It is also reminiscent of the increase in DNA synthesis that occurs throughout the remaining liver after partial hepatectomy.[13] In both of these latter situations it has been postulated that the drug or procedure has resulted in an effect being produced on the control mechanism of DNA synthesis in specific cell types of the affected organ. Intensive studies are underway in many laboratories in an attempt to clarify the cellular events accounting for the change. Similar studies seem indicated in order to clarify events in arterial SMC altered by cholesterol feeding.

One of the first things that needs to be known is what part or parts of the arterial SMC reproductive cycle has been changed. Figure 1 shows the basic features of the cell reproductive cycle as it is currently understood. In most tissues only a part of the total cell population is in the reproductive cycle at any one time. The other cells in the tissue, which would generally be those performing the specialized function of the tissue (e.g., contraction in SMC), have left the reproductive cycle. Some of these are prob-

TABLE III.—*Mitotic counts of aortic trifurcation region of swine fed cholesterol for three days, expressed as numbers of mitoses per 10^4 cells per hour*

	Cholesterol	Control	p value*
Endothelium	2.3	1.1	<.01
Subondothelial intima	3.2	1.5	<.01
Inner media	4.0	1.9	<.01

* Mann-Whitney U-test.

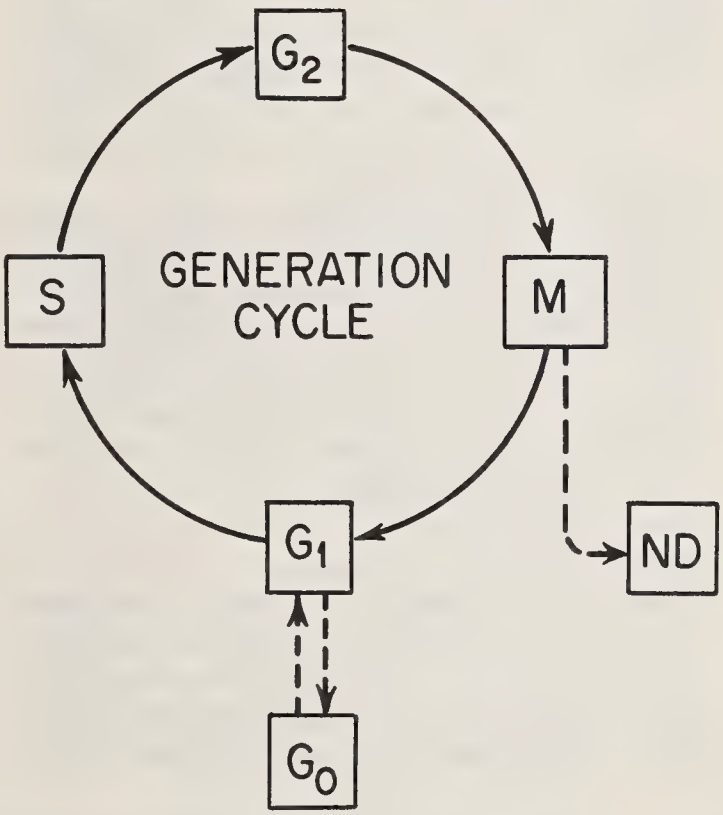


FIGURE 1.—The cell cycle. M = mitosis; G_1 = post mitosis—pre-DNA synthesis period; S = DNA synthesis period; G_2 = post DNA synthesis—premitosis period; G_0 = potential dividers that may be stimulated to re-enter the generation cycle; ND = nondividers that are permanently incapable of dividing.

ably unable to return under any circumstances. Others can probably re-enter if stimulated appropriately.

Therefore, a study was designed which had as its basic aim interpretation, in terms of the cell cycle, of the observations already described regarding the effect of mild cholesterol diets on DNA synthesis and division of arterial SMC. At the time these studies were begun very little was known about the nature and size of the dividing population of arterial SMC or their cell cycle (Table IV). For example, the dividing population might have been a small percentage of the total SMC that divided every few days (short generation time), retaining some progeny and releasing others into the non-dividing population. On the other hand, the majority of the arterial SMC, even though functioning in contraction, could have been in the dividing population, with DNA synthesis and division taking place only after long intervals in G_1 (long generation times) or G_0.[14] Another possibility that could also have resulted in long generation times was that a large proportion of the dividing population at any one time might have been in G_2, with divisions taking place only after a long sojourn.[15]

Obviously we needed to know which of these possibilities is correct before attempting to define the biochemical nature of the effect of cholesterol diets on arterial SMC reproduction kinetics. In particular we needed to know what group of SMC to investigate in future studies, i.e., (1) a small population dividing frequently, or (2) a large population in G_1 or G_0, or (3) a large population in G_2.

In one experiment 20 swine were given ^{3}H-thymidine intravenously at day 0. Ten were placed on the stock diet and 10 on the cholesterol diet. A pair consisting of one from each group was sacrificed at 2 hours and the remaining pairs sacrificed at 5-hour intervals up to 47 hours. All except the 2-hour pair (which were used to construct a generation table) were given colchicine 5 hours prior to sacrifice, thus providing sequential 5-hour harvests of labeled and unlabeled mitoses. Cross-sections of the aortic trifurcation were processed for autoradiography and grain counts per labeled cell recorded. Labeled and unlabeled mitoses were also counted. Only cells with 4 or more grains were considered to be labeled. The results of this experiment are summarized in Figure 2.

TABLE IV.—*Information available at outset of study on arterial SMC in relation to cell cycle*

Cells in:	S	~0.7%
	G_2	<1-98%
	M	<1%
	$G_1 + G_0$	<1-98%
	Dividing Population	2-98%
	Non-dividing population	0-98%
Generation time		?

The time curve of the percentage of labeled mitoses furnishes a method for estimating the duration of the different phases of the cell cycle.[16] The results in Figure 2 show that G_2 is at least 7 hours long, since no labeled mitoses were seen in the swine sacrificed at 7 hours after receiving ^{3}H-thymidine. We cannot estimate the duration of S in the usual manner because of the 5-hour time spread produced by colchicine. We can say that it is less than 15 hours, probably 8–10 hours (from another experiment currently in progress). The duration of S + G_2 + M is less than 37 hours because the curves (Figure 2) suggest that division of almost all of the labeled cells was complete by 37 hours. We checked this point by absorption microspectrophotometry of Feulgen-stained sec-

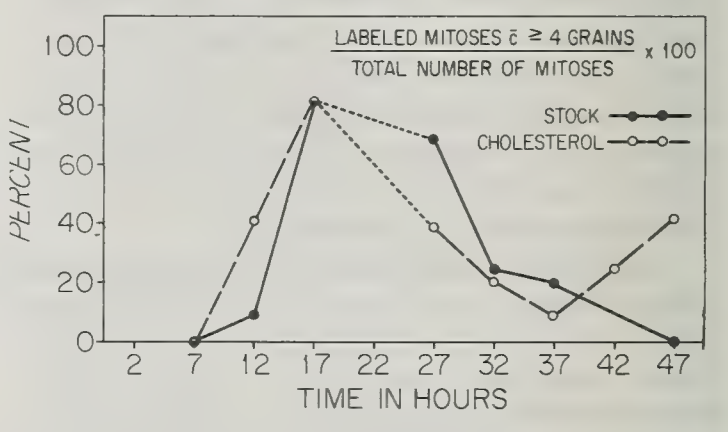


FIGURE 2.—Graphic presentation of data showing percentage of mitoses that had ≥ 4 grains in each time period. It is apparent that a wave of mitoses is completed by 37 hours, suggesting that the partial cycle time from S through M inclusive is no more than 37 hours. The graph also shows what appears to be the beginning of a second wave of mitoses in the cholesterol swine. Evidence is presented in the text and Figure 4 supporting the idea that the second wave represents the beginning of post-pulse labeling and not a second division of the labeled cells.

tions from the 37-hour stock and cholesterol diet-fed swine. The results showed that, in both groups, most of the labeled cells had the DNA content of diploid cells. This supports the finding that most labeled cells had divided shortly after synthesizing DNA and very few remained in G_2. No significant difference was found between the stock swine and cholesterol swine up to this period. By 42 hours, however, the cholesterol swine showed the beginning of a second peak of labeled mitoses. Although this could represent a second division of the labeled cells, another possibility is that it is due to post-pulse labeling. In *in vivo* situations, the availability time of ^{3}H-thymidine is generally no longer than 30–60 minutes, so that this can be considered a pulsing period. Labeling of cells at some time after this period can be referred to as post-pulse labeling. Post-pulse labeling is presumably due to re-utilization of label salvaged from dead cells in the intestine and elsewhere.[17]

In order to find out whether post-pulse labeling occurred, another experiment was carried out (Figure 3). A segment (No. 2) of the right carotid artery was surgically exposed and wrapped with parafilm and vascular clamps were applied at two points to isolate the segment from the general circulation during the period of pulsing with ^{3}H-thymidine. This technique was tested, and we found that the segment was completely isolated from the general circulation and that it was still capable of synthesizing DNA after a two-hour period. After the segment of right carotid artery was isolated, the swine were given ^{3}H-thymidine intravenously. Segments No. 1 and No. 3 were not isolated. Two hours later, long after completion of the pulsing period, the clamps were released so that any labeling of cells in the previously isolated segment would have to be due to post-pulse labeling. The swine were killed at 5 hours, 2 days, and 7 days. No post-pulse labeling was seen at 5 hours. However, at 2 days and 7 days there was considerable post-pulse labeling. Grain counts of the post-pulse labeled cells showed that most of them had less than 9 grains. In contrast, the non-isolated segments had heavily labeled cells.

Since most of the post-pulse labeled cells had

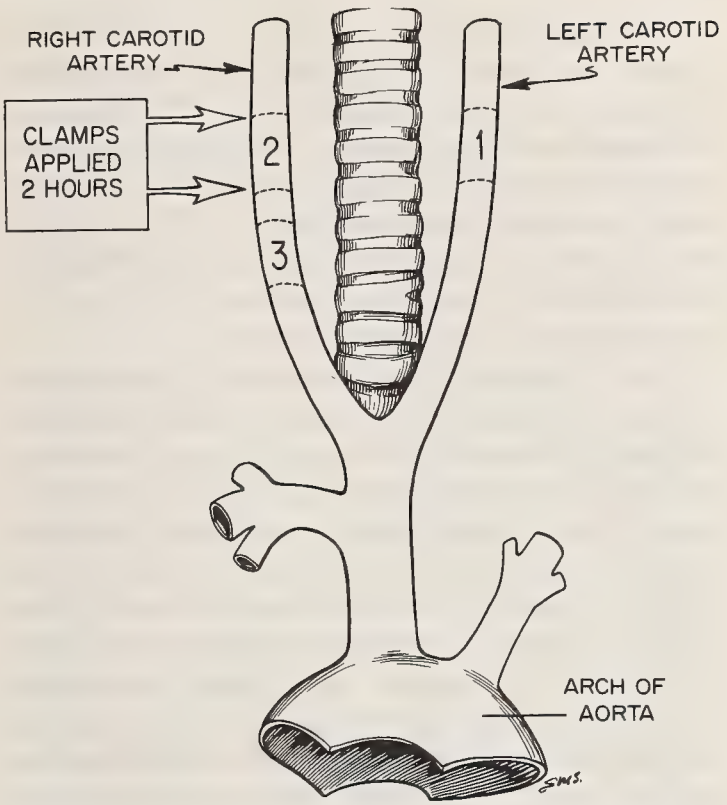


FIGURE 3.—Schematic drawing of carotid arteries illustrating where vascular clamps are applied and where sections are taken.

less than 9 grains, we reexamined the percentages of labeled mitoses using 9 grains as the "cut-off" point (Figure 4). When this was done, the second peak of labeled mitoses virtually disappeared. This suggests that it was due to post-pulse labeling and not to a second division

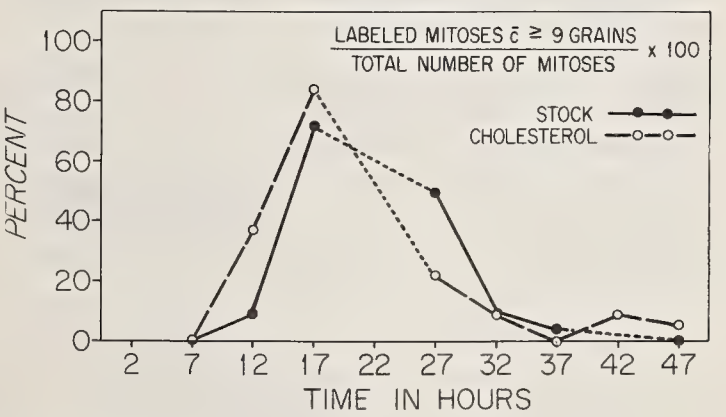


FIGURE 4.—Graphic presentation of percentages of labeled mitoses data using 9 grains as the "cut off" point since most of the post-pulse labeled cells had less than 9 grains. The second wave of labeled mitoses (Figure 2) virtually disappeared, indicating that it was due to post-pulse labeling and not to a second division of the labeled cells.

of the labeled cells, since a second division would not have resulted in most cells having less than 9 grains. Hence, the results of the labeled-mitoses experiment was consistent with no more than one division in 47 hours for both stock and cholesterol diet-fed swine. The generation time cannot be ascertained from this study, since it would be necessary to follow the labeled cells through two divisions.

The approach chosen in order to estimate the generation time was to pulse-label the cells with ^{3}H-thymidine and follow the grain count distribution of the labeled cells at different periods of time. Thirty-eight swine were given ^{3}H-thymidine at day 0. Two hours later, 8 swine were killed to provide the baseline grain count distribution, that is, the grain counts after the pulse labeling was completed but before the labeled cells had divided. The remaining swine were divided into two groups and fed either the stock or cholesterol diet. The swine were killed in pairs at 2 days, 7 days, 15 days, and 30 days. The tissue studied was cross-sections of the aortic trifurcation of the abdominal aorta. The specimens were processed for autoradiography and grain counts of labeled cells were made.

From the baseline grain count, a generation table such as that shown in Table V can be constructed. The numbers on this table are presented only as stylized illustrations and bear no relation to the actual experiment. The C_0 column represents the number of cells in the corresponding grain category listed in the first column.

When these cells divide, each daughter cell will have half as much ^{3}H-thymidine as the parent,[18] and at least in a statistical sense will produce half as many grains under standardized conditions. By utilizing this fact, the number of potential parents for each grain class in the next generation can be determined. For example, all of the cells with 16 grains are potential parents of cells with 8 grains.

However, these are not the only potential parents for cells with 8 grains, since cells in the original population with odd numbers of grains must also be accounted for; and these must be handled in a special way. For example, we can assume that in a statistical sense half of the cells with 15 grains at the outset will be parents of daughters with 8 grains and half of daughters with 7 grains. Likewise, half of the cells with 17 grains can be considered as potential parents of cells with 8 grains and half of cells with 9 grains. Thus the total available parents for cells with 8 grains will be all in the predivision population with 16 grains plus half of those with 15 and 17 grains, respectively.

The above process can be repeated with each grain class until the number of potential parents for each class in the next generation has been determined. This process can be used to construct a generation table for any number of generations that are desired.

TABLE V.—*Examples of construction of a generation table using cells observed after pulsing with ^{3}H-thymidine but before division*

No. of grains	C_0[a] No. of cells in each grain class after pulsing but before division	C_1 Cells in C_0 reclassified as potential parents	C_2 Cells in C_1 reclassified as potential parents
0-3		40	168
4	10 (5.0%)	40 (25.0%)	32 (100%)
5	10 (5.0)	40 (25.0)	
6	10 (5.0)	35 (21.9)	
7	20 (10.0)	20 (12.5)	
8	20 (10.0)	20 (12.5)	
9	20 (10.0)	5 (3.1)	
10	20 (10.0)		
11	20 (10.0)		
12	20 (10.0)		
13	10 (5.0)		
14	10 (5.0)		
15	10 (5.0)		
16	10 (5.0)		
17	10 (5.0)		
Total ≥ 4	200 (100%)	160 (100%)	32 (100%)

If one were dealing with a delimited cell population, such tables could be used to predict absolute numbers of labeled cells that would be present in each grain class after a selected number of divisions. In *in vivo* situations, however, one is seldom dealing with a delimited population. It is meaningless to ask which batch of cells in the aorta of experimental animal B corresponds to the batch counted in baseline animal A; the most one can say is that both batches were selected, as nearly as possible, from corresponding regions of the two organs. To compare predictions such as can be made from generation tables and observations made at some time after divisions have taken place, it is necessary to convert both predictions and observations to percentages as shown in this table. Also, when cells in the original population divide, some of them will have their grain numbers diluted by division so that they fall below the counting threshold. When we express our theoretical predictions of the grain count distribution after division, we correct the total number of cells for the predicted number that "disappear" below the threshold; and the percentages are obtained by dividing by this corrected total.

In an actual experiment, the predicted percentage with 4 grains after one division may be compared with the observed percentage with 4 grains. If the values are similar, we could say that the results with the 4-grain category are consistent with the hypothesis that one division has taken place in the observed population. If the other grain classes also are consistent with predictions for one division, we would consider that we had strong evidence for the occurrence of just one division in the labeled population. If, on the other hand, values predicted for one division and values observed are not similar, one would try other whole or fractional numbers of divisions until the best fit is achieved.

The results of the 30-day experiment are shown in Table VI. Analyses of the data were made in all grain categories. The results were similar for all categories, so that what is presented in this table is a simplified analysis of two grain classes, 14–23 and > 24. The observed values of the stock swine were similar to those of the cholesterol swine, indicating no difference in generation time between the two groups up to 30 days after the administration of ^{3}H-thymidine. It is also apparent that, for both groups, the best fit of the observed values among the predicted values was for one division. The results of the 2-day, 7-day, and 15-day experiments were also consistent with the occurrence of only one division. This is the division that took place shortly after the pulse-labeling period. From the labeled-mitoses experiment, we know that the initial division of the labeled cells was completed around 37 hours after the administration of ^{3}H-thymidine. Therefore, the generation time for both stock and cholesterol diet-fed swine is longer than 28 days.

Since most of the post-pulse labeled cells in the swine carotid artery of the previous experiment had less than 9 grains, we examined the 4–8 grain category of both stock and cholesterol groups. The results are presented in Tables VII and VIII. The values for the cholesterol group are significantly higher than those of the stock group in most periods of observation. This indicates that a greater number of cells is synthesizing DNA and being labeled in the post-pulse period among the cholesterol-fed swine than among the stock-fed swine. This suggests that more SMC enter S (the DNA synthesis part of

TABLE VI.—*Brain count data of all grain categories from the 30-day experiment**

Grain class	Stock (6) at 30 days (% observed)	Cholesterol (6) at 30 days (% observed)	% predicted after ½ divide once only and ½ do not divide	% predicted after all divide once only
14-23	53.9** (av. of 49.0, 53.4, 35.6, 49.5, 59.0, 77.1)	56.4** (av. of 42.6, 61.5, 73.0, 65.6, 50.7, 44.8)	40.0	55.3
≥24	46.1	43.6	60.0	44.7

* Comparison of predicted percentage of cells in 14- to 23-grain class among those left with ≥ 14 grains, after one half of cells have divided once and after all cells have divided once with observed percentage in two groups.

** Difference between stock and cholesterol not significant by Mann-Whitney U test.

TABLE VII.—*Grain count data of 4–8 grain category**

Days after ^{3}H-thymidine administration	Stock: Labeled cells c 4–8 grains/ total c ≥ 4 grains	Stock: %	Stock: Av % for group	Cholesterol: Labeled cells c 4–8 grains/ total c ≥ 4 grains	Cholesterol: %	Cholesterol: Av % for group	p value**
2	204/1022	20.0	20.8	311/1077	28.9	33.1	—
	212/981	21.6		430/1156	37.2		
7	327/1017	32.2		394/1002	39.3		
	358/1043	34.3	33.2	299/727	41.1	42.3	.05
	340/1031	33.0		314/674	46.6		
15	100/323	31.0		248/562	44.1		
	113/413	27.4	33.1	321/547	58.7	54.4	.05
	118/288	41.0		306/506	60.5		
30	215/522	41.2		418/539	77.6		
	359/722	49.7		718/973	73.8		
	354/905	39.1	44.7	622/1008	61.7	68.8	.001
	100/227	44.1		144/225	64.0		
	106/193	54.9		241/357	67.5		
	35/90	38.9		173/253	68.4		

* Percentage of labeled cells with 4–8 grains among total labeled cells with ≥ 4 grains.
** Mann-Whitney U test.

TABLE VIII.—*Labeling indices* of 4–8 grain category from grain count data*

Days after ^{3}H-thymidine administration	Stock: Labeled cells c 4–8 grains/ total cells	Stock: %	Stock: Av % for group	Cholesterol: Labeled cells c 4–8 grains/ total cells	Cholesterol: %	Cholesterol: Av % for group	p value**
2	204/23,750	0.87	1.00	311/52,810	0.59	1.14	—
	212/18,790	1.13		430/25,570	1.68		
7	327/28,750	1.14		394/23,920	1.65		
	358/30,140	1.19	1.15	299/26,710	1.12	1.46	Not significant
	340/29,440			314/19,440	1.62		
15	100/15,170	0.66		248/29,250	0.85		
	113/23,920	0.47	0.66	321/29,720	1.08	1.08	.05
	118/14,020	0.84		306/23,300	1.31		
30	215/17,100	1.26		418/13,050	3.20		
	359/16,930	2.12		718/20,670	3.47		.032
	354/18,420	1.92	1.05	622/26,062	2.39	2.35	
	100/13,680	0.73		144/22,200	0.65		
	106/46,710	0.23		241/9,110	2.65		
	35/54,160	0.06		173/9,930	1.74		

* Percentage of labeled cells with 4–8 grains among total cells, labeled or unlabeled.
** Mann–Whitney U test

the cycle) from G_1 or G_0 cells in the cholesterol group than in the stock group. This means that the time in G_1 or G_0 has been shortened.

The results of these experiments permit us to define at least in broad terms some of the elements of the cell cycle shown in Figure 1. We know from the labeled mitoses experiment that the duration S + G_2 + M is less than 37 hours. We cannot estimate S in the usual manner[16] from the labeled mitosis curves because of the 5-hour spread produced by colchicine. However, we can say that S is no more than 15 hours and probably less (preliminary results from a study in progress suggest an 8–10 hour S). G_2 is at least 7 hours, for the first labeled mitoses were seen in the swine given colchicine 7 hours after receiving ^{3}H-thymidine and sacrificed at 12 hours. There is no reason to believe that M is any longer than 1 or 2 hours. No evidence is presented indicating that any of these times differ significantly between the cholesterol swine and the stock swine.

The division observed during the first 2 days represents the completion of a partial generation, beginning with S. No evidence for a second division is apparent up to 30 days. Hence, the generation time of SMC in both controls and cholesterol animals must exceed 28 days. Thus the extent that the generation time of the cholesterol group has been decreased by choles-

terol diet can not be determined without more extended experiments designed to test this point.

The possibilities can be narrowed down, however. In a homogeneous population (no G_0 component) with uniform age distribution, the labeling index (ratio of cells in S to total cells in the population at any moment) is equal to the ratio of S time to generation time.[19] The labeling index of our tissues at the beginning of the experiment was about 0.7 percent. Taking the S time as 8 hours, we calculate a generation time of 48 days.

This value is maximal (if S is really 8 hours). The assumption of an exponential age distribution would result in multiplying the value of $\log_e 2 = 0.693$, giving 33 days.[19] If the population were not homogeneous, and contained a G_0 component, the denominator of the labeling index would be a fraction of the total population—that part of it in the cycle at a given moment. With growth fraction 2/3, the generation time would then be 32 days. An increasing proportion of G_0 phase (decreasing growth fraction) would bring the generation time into a range (less than 28 days) from which we can exclude it experimentally.

Granted the validity of our observations and an S of 8 hours, we can bracket the generation time between 28 and 48 days. If our preliminary estimate of 8 hours for S proves to be low, the calculated generation time would of course be longer. As to the generation time of the excess post-pulse labeled cells that have been recruited by the cholesterol diet, we can say nothing, and must await the results of observations of longer periods. The fact that mitoses were numerous among these cells at 2 and 7 days (that is, during the period of post-pulse labeling) might plausibly be expected, and the fact that they were negligible at 15 days and 30 days suggests that these cells, like the original pulse-labeled group, have generation times greater than 28 days.

Because of the limited time of observation, we can do no more than guess as to whether the newly recruited cells were derived from G_0 or resulted from acceleration of cells slowly meandering through G_1. If longer periods of observation indicate that the original pulse-labeled cells are no faster than they were initially, the decision will be clear. Moreover, knowledge of the generation time of this population would permit us, by calculations similar to those just discussed, to estimate the magnitude of G_0 (which evidently might be negligible). As to a non-dividing population, our experiments give no clue as to whether it even exists in aortic SMC, let alone what its properties might be. In Table IX a summary is given of some of the data we now know or can surmise regarding distribution of arterial SMC in relation to the cell cycle. Clearly the cells in G_1 or G_0 are the ones on which we now need to focus our attention.

It is interesting to note that the effect of most other known stimulators of DNA synthesis is on G_1 or G_0 cells. These include the effect of partial hepatectomy on hepatic cells[13] and of isoproterenol on the salivary glands.[12] The biochemical mechanisms that account for the stimulation are not known for any type of stimulus. With most, either a rise in RNA synthesis has been the first change observed or the effect has been abolished by small doses of actinomycin D.[20] This has led many investigators to postulate that the stimulatory effect was on transcription. The postulated effect could be produced either by removal of an inhibitor that is normally present or through some direct interaction. RNA synthesis and the effect of actinomycin D have not yet been investigated in cholesterol-fed swine.

Our attention is now turning to investigation of mechanisms that control whether or not an arterial SMC is going to synthesize DNA and divide. We have evidence from *in vitro* studies suggesting that there is something in the serum of hypercholesterolemic swine that is involved in control mechanisms.[21] A greater proportion of cultured arterial SMC are synthesizing DNA

TABLE IX.—*Information now surmised on arterial SMC in relation to cell cycle*

		Outset	Now
Cells in:	S	~0.7%	~0.7%
	G_2	<1-98%	<1%
	M	<1%	<1%
	$G_1 + G_0$	<1-98%	50-98%
	Dividing population	2-98%	50-100%
	Non-dividing population	0-98%	0-50%
Generation time		?	>28 days

TABLE X.—*^{3}H-thymidine labeling indices of swine aortic tissue culture cells grown in 20% autologous serum*

Group*	Labeling Index (%)	P values**	
StSs	21.5	StSs<StCs	$p < .001$
StCs	30.7	StSs<CtCs	$p < .001$
CtSs	22.5	CtSs<CtCs	$p < .001$
CtCs	31.4		

* StSs = Tissue from stock diet fed swine grown in serum from stock diet-fed swine; StCs = Tissue from stock swine in serum from cholesterol swine; CtSs = Tissue from cholesterol swine in serum from stock swine; CtCs = Tissue from cholesterol swine in serum from cholesterol swine.

** Chi square test.

at any given time when the cells are grown in hypercholesterolemic serum than when grown in control serum. One of these experiments is illustrated in Table X.

In studies from other laboratories of skin, liver, and a few other tissues, evidence has been presented suggesting that normal plasma contains a cell-type-specific substance that inhibits cell multiplication in the target cell. In a few situations in which rate of cell multiplication is increased (for example, after partial hepatectomy) the available evidence from both *in vivo* and *in vitro* studies suggests that the amount of the cell-type-specific inhibitory substance is reduced in the blood and in the affected tissue. We have just begun experiments designed to determine whether or not such a substance exists for arterial SMC but do not as yet have sufficient results to warrant conclusions.

SUMMARY

A series of studies of arterial wall metabolism in cholesterol-fed swine has been presented. These show that there is increased metabolic activity from the earliest days on diet. The most significant manifestation from the standpoint of atherogenesis appears to be the excessive number of arterial SMC synthesizing DNA and dividing. From an overall point of view, the goal of all of the studies that were presented is to find an acceptable means to control or prevent the development of the clinical manifestation of atherosclerosis in man. It is at least within the realm of possibility that this goal might be achieved by learning how to control and prevent the excessive proliferation of arterial SMC that occurs so commonly in modern man.

REFERENCES

1. THOMAS, W. A., FLORENTIN, R. A., NAM, S. C., KIM, D. N., JONES, R. M., and LEE, K. T. Preproliferative phase of atherosclerosis in swine fed cholesterol. Arch. Pathol. 86:621–643, 1968.
2. FLORENTIN, R. A., and NAM, S. C. Dietary-induced atherosclerosis in miniature swine. I. Gross and light microscopy observation: Time of development and morphologic characteristics of lesions. Exp. Mol. Pathol. 8:263–301, 1968.
3. SCOTT, R. F., JARMOLYCH, J., FRITZ, K. E., IMAI, H., KIM, D. N., and MORRISON, E. S. Reactions of endothelial and smooth muscle cells in the atherosclerotic lesion. In *Atherosclerosis: Proceedings of the Second International Symposium* (R. J. Jones, ed.), Springer-Verlag, New York, pp. 50–58, 1970.
4. FLORENTIN, R. A., NAM, S. C., LEE, K. T., LEE, K. J., and THOMAS, W. A. Increased mitotic activity in aorta of swine after three days of cholesterol feeding. Arch. Pathol. 88:463–469, 1969.
5. SCOTT, R. F., MORRISON, E. S., and KROMS, M. Aortic respiration and glycolysis in the pre-proliferative phase of diet-induced atherosclerosis in swine. J. Atheroscler. Res. 9:5–16, 1969.
6. MORRISON, E. S., SCOTT, R. F., KROMS, M., and PASTORI, S. J. A method for isolating aortic mitochondria exhibiting high respiratory control. Biochem. Med. 4:47–54, 1970.
7. SKINNER, F., SCOTT, R. F., and MORRISON, E. (Personal communication).
8. WHEREAT, A. F. Fatty acid synthesis in cell-free system from rabbit aorta. J. Lipid Res. 7:671–677, 1966.
9. KIM, D. N., LEE, K. T., and THOMAS, W. A. Dietary induced atherosclerosis in miniature swine. IV. Metabolic studies: In vitro protein synthesis by aortic strips from swine fed atherogenic diets. Exp. Mol. Pathol. 8:263–301, 1968.
10. IMAI, H., and THOMAS, W. A. Cerebral atherosclerosis in swine. Role of necrosis in progression of dietary induced lesions from proliferative to atheromatous stage. Exp. Mol. Pathol. 8:330–357, 1968.
11. IMAI, H., LEE, K. J., LEE, S. K., O'NEAL, R. M., and THOMAS, W. A. Degeneration of arterial smooth muscle cells: Ultrastructural study of smooth muscle cell death in control and cholesterol-fed swine. Virchows Arch. Abt. A. Path. Anat. 350:183–204, 1970.
12. BASERGA, R. Biochemistry of the cell cycle. A review. Cell Tissue Kinet. 1:167–191, 1968.
13. FUJIOKA, M., KOGA, M., and LIEBERMAN, I. Metabolism of ribonucleic acid after partial hepatectomy. J. Biol. Chem. 238:3401, 1963.
14. LAMERTON, L. F. Cell population kinetics in relation to homeostasis. In *Homeostatic Regulators* (G.E.W. Waterholme and J. Knight, eds.), Churchill, London, pp. 5–28, 1969.
15. PELC, S. R. Incorporation of labeled precursors of DNA in non-dividing cells. In *Cell Proliferation*

(L. E. Lamerton and R. J. M. Fry, eds.), Davis, Philadelphia, 1963.

16. WIMBER, D. E. Methods for studying cell proliferation with emphasis on DNA labels. In *Cell Proliferation* (L. F. Lamerton and R. J. M. Fry, eds.), Davis, Philadelphia, pp. 1–17, 1963.
17. BASERGA, R., and KISIELESKI, W. E. Comparative study of the kinetics of cellular proliferation of normal and tumorous tissues with the use of tritiated thymidine. I. Dilution of the label and migration of labeled cells. J. Nat. Cancer Inst. 28:331–339, 1962.
18. TAYLOR, J. H., WOODS, P. S., and HUGHES, W. L. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. Nat. Acad. Sci. U.S.A. 43:122–128, 1957.
19. CLEAVER, J. E. Thymidine metabolism and cell kinetics. American Elsevier, New York, 1967.
20. LIEBERMAN, I., ABRAMS, R., and OVE, P. Changes in the metabolism of ribonucleic acid preceding the synthesis of deoxyribonucleic acid in mammalian cells cultured from the animal. J. Biol. Chem. 238:3955, 1963.
21. FLORENTIN, R. A., CHOI, B. H., LEE, K. T., and THOMAS, W. A. Stimulation of DNA synthesis and cell division in vitro by serum from cholesterol-fed swine. J. Cell Biol. 41:641–645, 1969.

DISCUSSION

CHAIRMAN KUSSEROW: Thank you, Dr. Thomas. I think after hearing Dr. Thomas, we will start worrying the moment that we swallow the next glass of milk, if things start as early as he says.

KEITH MCCULLAGH, Cleveland Clinic: I wonder, Dr. Thomas, whether you can speculate as to the cause of the increase in protein synthesis, oxygen uptake, and everything else. Is the lipoprotein, which is infiltrating the wall, directly stimulating these events, or is it an indirect effect of cell death where the necrotic cell is releasing an activating factor?

DR. THOMAS: Well, that is certainly an interesting question that goes right to the heart of the matter. One way of looking at it is that we are simply dealing with injury. In some unknown way, the cholesterol diet is damaging vessels, then all we see is repair, more damage, and more repair, and so on down the line. That's a hypothesis. I liked it better myself before we found the increase in the number of cells that were dividing so very, very early. I would like to have seen more injury followed by the increase than we saw, but we haven't found it that way so far. We know, however, that with any type of injury, repair starts almost immediately. So that doesn't rule it out.

You asked for speculation, so I'll speculate on another thing. There are some interesting "substances" that have been described as regulating the rate of multiplication of cell growth in skin and a number of other tissues which are sometimes referred to as chalones. They haven't been identified well enough really to glorify them with a name. But whatever it is, there is a property of tissue extract (skin for example) that will decrease the rate that cells divide or synthesize DNA in some animals. This is one speculation that we've made in our own laboratory. There may be a substance normally present that is involved in the control of the division rate of arterial smooth muscle cells. Then somehow or other, the cholesterol changes in the blood interfere with this action and allows the cell to divide faster. We've been looking for this substance, and we think we have found it. That doesn't prove the theory; it is a speculation.

DR. BLACKSHEAR: Did you look at the oxygen saturation curve with and without high cholesterol to see what the P450 shift was in the two cases?

DR. THOMAS: The oxygen uptake studies, studies of isolated mitochondria and so on, were done in my laboratory by other people. I'm afraid I can't answer that. Mitochrondrial function was one thing we thought might be wrong. Others have suggested that there was an uncoupling or a partial uncoupling of the mitochondria which might lead to some of these events, but we have not been able to see that. The mitochrondria in vitro appear to be as well-coupled as they are in the controls.

CHAPTER X

GENETIC AND BIOLOGICAL EFFECTS OF RADIATION

Radiation Studies with Swine

Extracorporeal Irradiation of Blood Lymph

Preliminary Data on Chromosome Aberrations in Swine and Human Leukocytes with a Brief Review of Factors Affecting their Observed Frequency

Biological Effects of Radiation from Simulated ^{238}Pu Power Sources in Dogs

Observations on Chromosomal Morphology During Simulated Intracorporeal Radiation from Nuclear-Powered Artificial Hearts

Pathologic Effects of Chronic ^{90}Sr Ingestion in Miniature Swine

A Four-Year Study of Long-Term Effects of Implanted Discrete Heat and Radiation Sources in Dogs and Primates: Preliminary Studies Leading to Implantable Nuclear-Fueled Circulatory Support Systems

Animal Radiation Data and their Relevance to Man

RADIATION STUDIES WITH SWINE

D. G. Brown*

Swine are one of the most popular species among large animals in biomedical research. In the field of radiation biology, more data are available on swine than on other large domestic animals. The $LD_{50/30}$ values reported for swine range from 225 to 710 rads (tissue dose in air). These values reflect the influence of a number of variables such as dose rate, type and quality of radiation, age, size, and breed of swine. The ability of swine to recover from irradiation injury has been demonstrated by relative effectiveness of dose rate, by repeated exposures in the $LD_{50/30}$ range, and especially by the relatively large accumulated gamma radiation doses when given in fractions of 50 and 100 R/day to mortality. Although swine appear to have the highest rate of recovery from radiation injury among the large animal species, the early clinical syndrome and pathological manifestations were similar to those of other large animals.

Late effects observed in swine after exposure to bomb radiation were an increase in the incidence of genital, intestinal and hepatic neoplasms and a decrease (3% per 100 rads) of life span. Neoplasms have not been a factor relative to late effects of irradiation in mortality of cattle and burros.

INTRODUCTION**

The intent of this paper is to review the general response of swine after total-body exposure to X- and gamma-radiation and to mixed neutron-gamma radiation. Data reported by scientists from other laboratories will be included in this report, but the information presented will be comprised principally of data compiled at the UT–AEC Agricultural Research Laboratory. I shall deal specifically with dose-mortality interrelationships and with variables having an apparent effect on the end results. Consideration will be given to both early and late effects.

* Agricultural Research Laboratory of the University of Tennessee, Oak Ridge, Tennessee.

** This manuscript is published with the permission of the Dean of the University of Tennessee Agricultural Experiment Station, Knoxville. Operated by the Tennessee Agricultural Station for the U.S. Atomic Energy Commission under Contract No. AT-40-1-GEN-242.

EARLY MORTALITY

There is a rather large range of $LD_{50/30}$ values published for swine (Table I). This range of values is undoubtedly due primarily to a number of variables which were different in each experiment. There was also the problem of possible variable dosimetry among the laboratories.

The radiation variables which seemed to influence considerably the 30-day mortality of swine were type of radiation, exposure aspect (unilateral and bilateral) and dose rate. Biologic variables which appeared to be of significance were genotype, age, and size. The interrelationship of radiation and biologic variables complicates interpretation of effects of a specific variable; therefore, one can evaluate a variable only with reservation.

Type of Radiation

The relative biological effect (RBE) for neutrons when compared with gamma or X-rays in causing 30-day mortality appears to be approximately 1. Values of RBE will vary considerably with the dose parameters used for comparisons (Table II).

The average survival time of swine after single exposures in the $LD_{50/30}$ range was approximately 14 days for either neutron or photon radiation.[1–7,9] The cause of death within 30 days was usually associated with damage to the bone marrow (hemorrhagic syndrome), but the general behavior of the swine varied with type of radiation. Swine exposed to gamma radiation were usually active during the first 10 days after exposure with only a slight decrease in ingestion of food and water. Swine irradiated with fission[6] or 14–MeV neutrons[7] were depressed and lethargic during the first week with a pronounced decrease in consumption of food

TABLE I.—*Thirty-Day Mortality of Swine after Exposure to Ionizing Radiations under Various Experimental Conditions*

Source	Method of exposure	Dose Rate	$LD_{50/30}$		Age (Months)	Av. Wt. (kg)	Ref. No.
			In air.	Midline, rads			
X-ray, 2000 Kvp	Unilateral	15 R/min	500 R	300*	4.5–6.5	62	3
X-ray, 2000 Kvp	Bilateral	15 R/min	350–400 R	223*	4.5–6.5	62	3
X-ray, 1000 Kvp	Bilateral	30 R/min	510 R	242*	—	50–104	3
Bomb, γ	Unilateral		230 R	185*	4.6	30	8
X-ray, 1 Mvp	Bilateral	9–10 R/min	399 R	262*	8–9	110	2
^{60}Co, γ	4 π field	18–29 R/min	335 R	211*	—	34	5
^{60}Co, γ	4 π field	18–29 R/min	393 R	221*	—	68	5
^{60}Co, γ	Multisource**	0.85 R/min	618 R	359*	6	75	1
^{60}Co, γ	Multisource**	1 rad/min	710 rads	369	8	114	3
^{60}Co, γ	Multisource**	10 rads/min	435 rads	226	8	114	9
^{60}Co, γ	Multisource**	50 rads/min	360 rads	187	8	114	9
Bomb, n-γ	Unilateral		486 rads	—	4	38	4
Fission Neutrons	Unilateral	16–20 rads/mins	869 rads	370	9–12	150	6
Fission Neutrons	Bilateral	16–20 rads/mins	820 rads	350	9–12	150	6
14 Mev Neutrons	Bilateral	10 rads-min	585 rads	525	8	90	7

*Calculated from data contained in the respective reports as referenced.
**Predominately bilateral.

TABLE II.— *Calculated $LD_{50(30)}$ Doses for ^{60}Co Gamma Rays, Fission Neutron and 14-MeV Neutrons and Estimated RBE's for Neutrons. Dose Rates Were Approximately 10 Rads/Minute for Gamma Rays and 14-MeV Neutrons and 18 Rads/Minute for Fission Neutrons**

	^{60}Co, Gamma	Fission Neutrons	14 MeV Neutrons	RBE	
				^{60}Co/ Fission Neutrons	^{60}Co/ 14 MeV Neutrons
Tissue dose in air (rads)	435	820	585	.5	.7
Midline tissue dose (rads)	225	263	457	.9	.5
Av. tissue dose (rads)**	265	370	415	.7	.6
Av. bone marrow dose (rads)	281	385	415	.7	.7

*Brown and Haywood.[7]
*Average dose across the abdomen.

and water. The degree of change was greatest in the swine irradiated with 14–MeV neutrons. Constipation was common in the swine irradiated with 14–MeV neutrons, but was not evident in the swine irradiated with either fission neutrons or gamma rays. These differences were indicative of the degree of damage to the gastrointestinal tract. Pathologic alterations in the skin were severe only in the swine irradiated with fission neutrons.

Exposure Aspect

Nonuniform radiation doses cause variation in the dose-mortality response of swine. This has been demonstrated by bilateral and unilateral irradiation with X-rays[3] and neutrons.[6] Such variations contribute to the enigma of expression of radiation dose relative to effects on animals. Various dose parameters (average tissue dose, midline tissue dose, etc.) have been used, but none have proven completely satisfactory in comparative analyses. A method has been proposed for evaluating nonuniform exposures on the basis of dose to the bone marrow.[10] Dose-survival curve for stem cells in swine must be established, however, before this method will have merit over average tissue dose in this species.[6]

Dose Rate

Another variable affecting an animal's response to a dose of photon radiation is the time over which the dose is delivered. Early mortality was enhanced by an increase in dose rate in small laboratory animals.[11–13] This phenomenon has been tested on swine. Duroc swine, approximately 8 months old having an average weight of 114 kg, were exposed to ^{60}Co gamma radiation at dose rates of 1, 10, and 50 rads per minute (tissue dose in air).[9] The $LD_{50/30}$ values

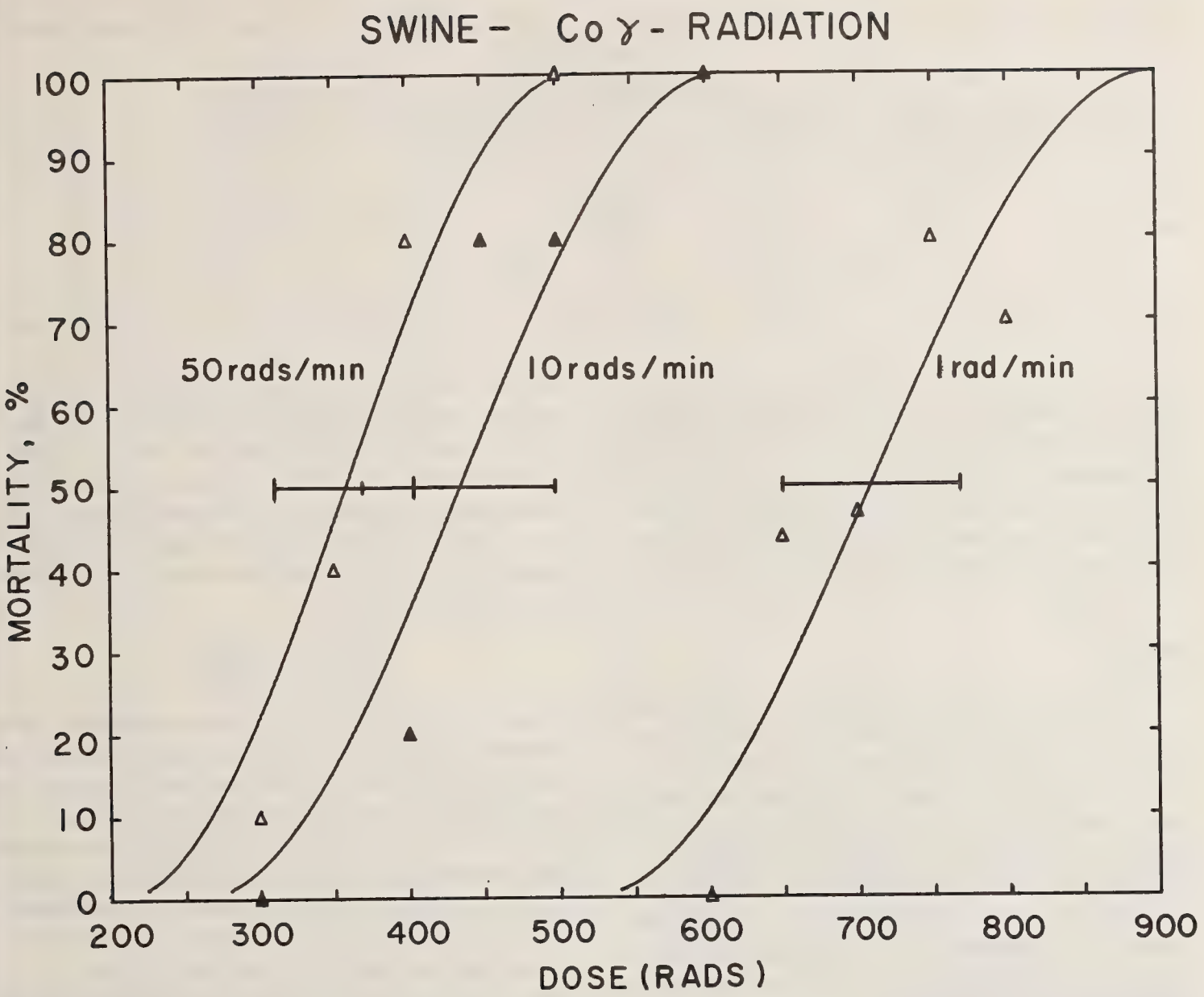


FIGURE 1.—Thirty-day mortality curves for swine exposed to ^{60}Co gamma radiation with 95% confidence intervals for 50% mortality.

were 710, 435 and 360 rads, respectively (Figure 1). The relative effectiveness of 10 and 50 rads per minute in causing 30-day mortality was 1.63 and 1.97, respectively, with 1 rad per minute having been arbitrarily assigned the value of 1. Although dose rate significantly affected the dose-mortality curves, it did not affect survival time or the general clinical syndrome.

The ability of an animal to recover from radiation injury seemingly is an important factor in the over-all response of an animal to irradiation. Swine appear to have the highest rate of recovery among the large animal species.[14] In studies where fractionated doses of gamma radiation were used (Table III), the apparent rate of recovery from radiation injury based on survival time seems unrealistic when compared with other large animal species, yet the data available are sufficient to be convincing. Recovery rate appears to vary among the swine[14,16,17] which is indicated in the mortality pattern from daily dose fractionation (Figure 2).

Recovery from radiation injury measured by the split-dose technique indicated that Duroc swine 8 to 9 months old had recovered from 51% of the initial injury at 3 days after exposure and from 65%, at 7 days. At 20 days after

TABLE III.—*The Mortality and Survival Time of Burros and Swine Exposed to Daily Doses of ^{60}Co Gamma Radiation until Death*

Daily Dose (R)	No. of Animals	Exposure Rate (R/min.)	Av. Weight (Kg)	Mean Surv. Time (days)	Mean Cumulative Lethal Dose (R)	Ref. No.
Swine						
100	60	0.55	58	22	2,220	16
100	32	0.47	45	56	5,600	17
50	10	0.55	56	205	10,250	26
Burros						
100	10	0.8	180	23.3	2,330	27
50	10	0.8	180	30.3	1,570	28
25	10	0.8	180	63.0	1,575	28

conditioning exposure the $LD_{50(30)}$ had increased by a factor of 1.64 (Table IV) above that for nonconditioned swine.[2] The apparent radioresistance was evident at 61 days and 107 days after exposure. Recovery from radiation injury in sheep measured by the same technique was approximately 70% at 30 days after irradiation.[15]

Genotype

The influence of genotype on the response of animals to irradiation has long been recognized.[18–21] From 2 experiments involving three breeds of swine exposed to daily doses of 100 R until death,[16] it was concluded that the mortality response (Figure 3) was influenced by genetic and physiologic factors other than those associated with breed definition. Environmental factors and nutrition in early life may contribute substantially to the variation in the mortality curve for swine.

Age and Size

The high correlation between age and size of swine less than 1 year old limits independent interpretation of either of these variables relative to influence on radiation response. The $LD_{50/30}$ for young pigs appears to be less than that for swine 8 to 9 months old,[22] but the data are not adequate for valid comparisons. In view of the range of $LD_{50/30}$ listed for various experiments (Table I), it is of interest to note the similarities in data from different laboratories for swine of the same breed, age and size exposed to X-rays and gamma-rays at dose rates of similar magnitude.[2,9]

TABLE IV.—*Thirty-Day Mortality of Swine before and after Conditioning Exposure (CE) to 1 Mvp X-rays**

CE (R)	Days after CE	$LD_{50/30}$ (R)	Recovery %	Mean Survival Time of Decedents (days)
0	—	399	—	15.6
240	3	282	51	16.3
265	7	306	65	13.3
265	20	654	196	11.3

*Nachtwey, Ainsworth, and Leong.[2]

LATE EFFECTS

Late effects of mixed gamma-neutron and X-irradiation were studied in swine throughout their life span.[23] The swine were exposed to bomb radiation at 4 months of age ($LD_{50/30}$ was 486 rads).[4] Thirty percent of the swine were survivors of a second radiation dose of X-rays (350 rads plus)[24] when they were approximately 8 months old. The total combined dose for the swine ranged from 15 to 850 rads (Table V).

Visible clinical changes were similar in the irradiated and control swine except for apparent unilateral atrophy of the musculature (Figure 4) in some of the irradiated individuals

TABLE V.—*Number of Swine per Dose Exposed to Bomb Radiation and X-rays*

Dose (rads)	Number of Swine		
	Total	Males	Females
Controls <1	35	23	12
15	8	5	3
75	7	3	4
250	10	5	5
380	9	7 (1)*	2
425	10	4	6 (3)
480	5	2	3
535	5	5 (1)	0
600	7	5 (5)	2 (2)
730**	10	5 (4)	5 (5)

*( )—Number of swine reirradiated with X-rays 4 months after bomb exposure. X-ray dose was approximately 68% of total dose.

**Mean dose (range 695 to 850 rads).

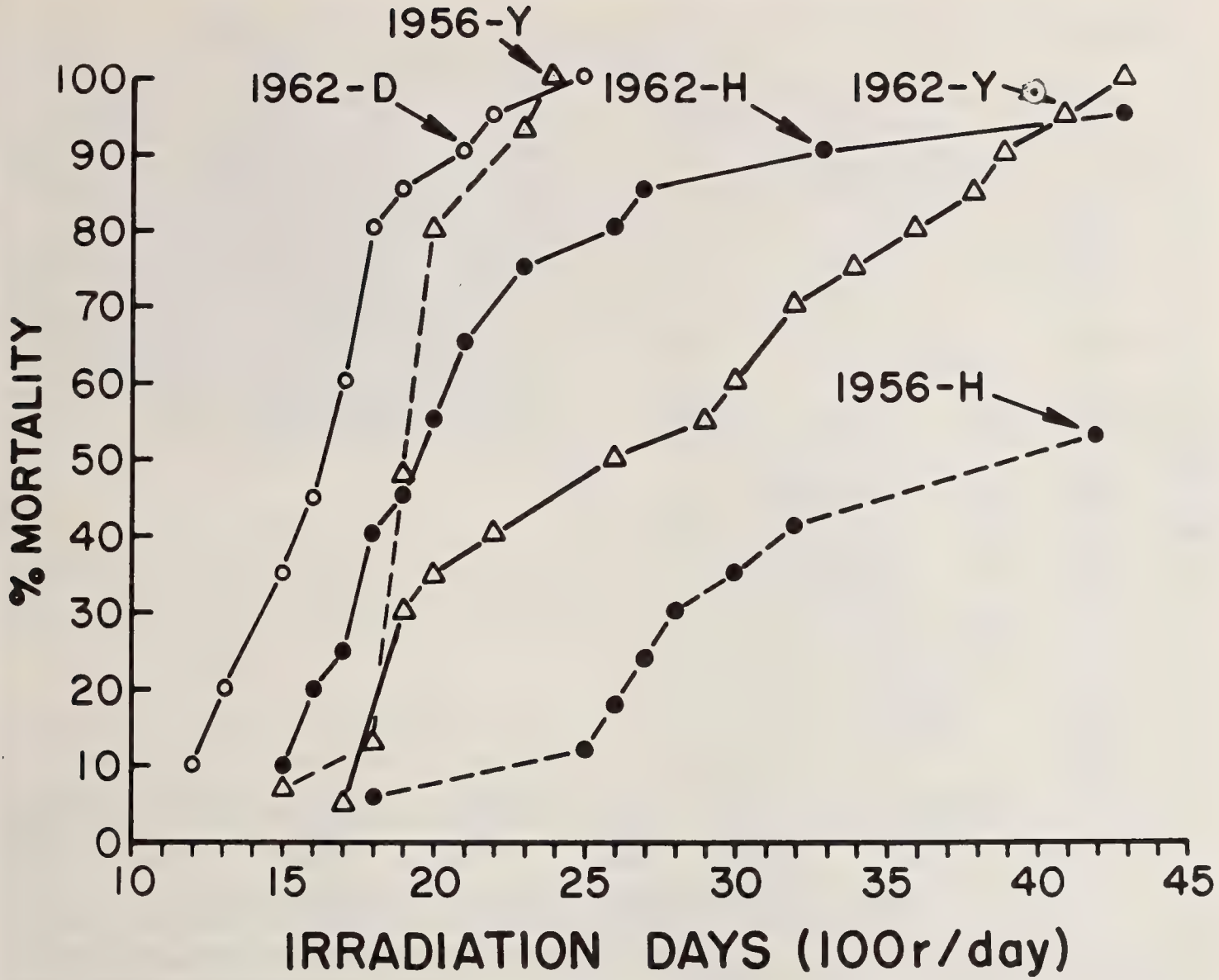


FIGURE 2.—Mortality in 3 breeds of swine (D = Duroc, H = Hampshire, and Y = Yorkshire) exposed to whole-body ^{60}Co gamma radiation in daily doses of 100 R until death. Each curve is identified by years in which data were obtained.

surviving a dose of bomb radiation above 250 rads. The major causes of death were chronic diseases.

A number of pathological changes were found in both irradiated and control[25] swine without any significant difference in frequency of occurrence between the two groups. Included among these aberrations were the following: gastroenteritis, intervertebral ankyloses and vertebral exostoses, degenerative arthritis, atherosclerosis in the abdominal portion of the aorta, dermatitis at a few sites in older swine, renal cysts, nephritis, pyogenic infections at various sites, urocystitis, cholecystitis, and endometritis. Evidently these and some other pathological manifestations were predominantly caused by trauma, infection, senility, or combinations of these factors.

Lesions attributable to effects of radiation were the following: asymmetrical diminution in the respective sizes of the musculature and underlying bone (Figure 5) in the pelvic region, usually in one pelvic limb; genital neoplasia, commonly manifested as leiomyomas of the broad ligaments and uteri; hepatomas; and annular, sclerosing adenocarcinomas usually present as stricture in the posterior one-third of the small intestines (Figure 6). The number of ani-

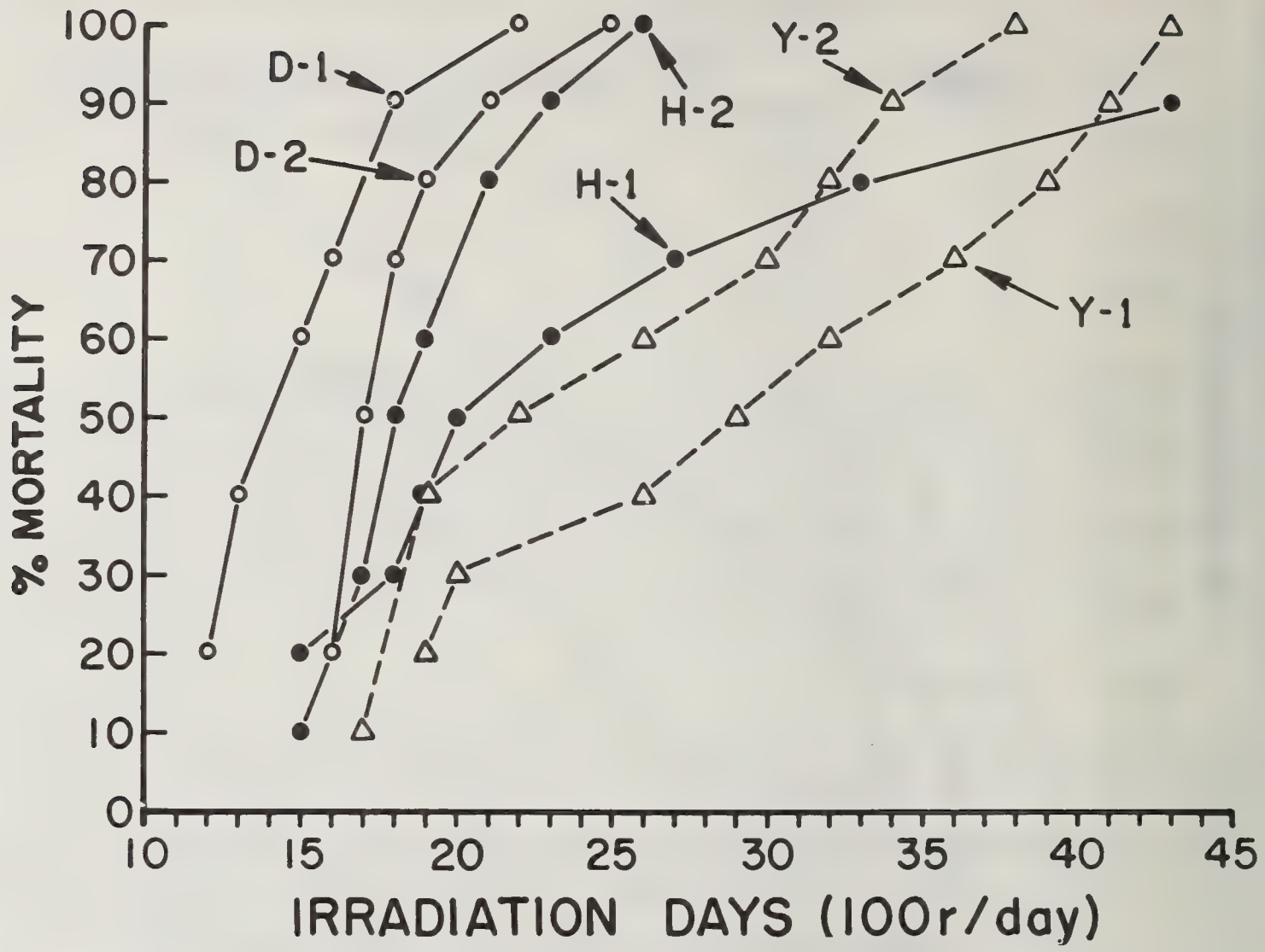


FIGURE 3.—Mortality in 2 breeding lines within 3 breeds of swine (Duroc, D–1 and D–2; Hampshire, H–1 and H–2; and Yorkshire, Y–1 and Y–2) exposed to whole-body ^{60}Co gamma radiation in daily doses of 100 R until death.

mals affected with genital, intestinal, and hepatic tumors was relatively higher in the irradiated swine than in the controls (Table VI). These tumors occurred earlier in the irradiated swine (Figure 7), this fact suggesting temporal advancement due to irradiation. No tumors were found in the controls less than 9 years of age except one animal affected with an ovarian tumor. The relatively immense size and weight (up to 27 kg) of the genital tumors in the irradiated swine contributed directly to deaths. These tumors were quite small in the controls and were not of significance relative to causes of deaths. The intestinal adenocarcinomas were in most instances important factors in the deaths of the swine so affected. The hepatomas presumably were not significant etiologic agents in the deaths of any of these swine. The specific cause of death was frequently difficult to identify. This was especially true in the aged swine

TABLE VI.—*The Number of Irradiated and Control Swine Affected with Genital, Intestinal, and Hepatic Tumors*

	No. of Animals	No. Subjected to Postmortem Examination	Tumors		
			Genital	Intestinal	Hepatic
Controls					
Males	23	23	1*	0	1
Females	12	11	7**	0	0
Irradiated					
Males	41	41	0	8	13
Females	30	28	23	6	5

*Urethral neoplasm.

**Six animals had tumors of the uteri and/or broad ligaments; one animal had an ovarian tumor.



FIGURE 4.—A swine exposed unilaterally (left side) to 550 rads of bomb radiation. The animal was 4 months old at time of irradiation. Unilateral atrophy of the tissues in the hip and thigh was a common lesion in those swine exposed to doses exceeding 250 rads of bomb radiation.

with the more numerous pathological changes often masking the primary cause(s) of death. The major causes of death were chronic diseases. Less than 25% of these swine died of acute and peracute diseases.

The mean postirrediation survival time for the irradiated swine (all doses combined) was 8.3 years for the males and 7 years for the females; and, for the control swine, 9 years for the males and 7.9 years for the females. Regression of survival time on dose (Figure 8) indicated a significant life-shortening effect on the females ($P < 0.05$); however, the regression coefficient for the males could not be declared different from zero. The effect on the females might have been related to the high incidence of genital tumors occurring earliest in and contributing to the deaths of the females in the highest dose groups. Mortality in the irradiated males was more erratic in relation to dose. The data of both sexes combined indicate that life shortening was approximately 3% per 100 rads.

SUMMARY

The $LD_{50/30}$ values for swine exposed to ionizing radiation were presented. The range of values were discussed with emphasis on specific variables which influenced the dose-mortality response.

Late effects of mixed gamma-neutron (bomb radiation) and X-irradiation were studied in swine throughout their life span. Neoplasms occurred earlier with a higher frequency in the irradiated swine than in the nonirradiated controls. Life shortening due to irradiation was approximately 3% per 100 rads.

REFERENCES

1. RUST, J. H., TRUM, B. F., WILDING, J. L., SIMMONS, C. S., and COMAR, C. L. Lethal dose studies with burros and swine exposed to whole-body cobalt-60 irradiation. Radiology 62:569–574, 1954.
2. NACKTWEY, D. S., AINSWORTH, E. J., and LEONG, C. F. Recovery from radiation injury in swine as evaluated by split-dose technique. Radiat. Res. 31:353–367, 1967.
3. TULLIS, J. L., CHAMBERS, F. W., JR., MORGAN, J. E., and ZELLER, J. H. Mortality in swine and dose distribution studies in phantoms exposed to supervoltage Roentgen radiation. Amer J. Roentgenol., Radium Ther. Nucl. Med. 67:620–627, 1952.
4. WOODWARD, K. T., MCDONNEL, G. M., HARRIS, P. S., KIRKLAND, W. J., and SHIVELY, J. N. The response of swine after exposure to the gamma-neutron flux of a nuclear detonation. Amer. J. Roentgenol., Radium Ther. Nucl. Med. 85:179–185, 1961.
5. CHAMBERS, F. W., JR., BILES, C. R., BODENLOS, L. J., and DOWLING, J. H. Mortality and clinical signs in swine exposed to total-body cobalt-60 gamma irradiation. Radiat. Res. 22:316–333, 1964.
6. BROWN, D. G., JOHNSON, D. F., and AUXIER, J. A. Unilateral and bilateral exposure of swine to fission neutrons. Health Phys. 21:537–545, 1971.
7. BROWN, D. G., and HAYWOOD, F. W. 14 MeV neutron irradiation of swine. (Unpublished data).
8. TULLIS, J. L., LAMSON, B. C., and MADDEN, S. C. Mortality in swine exposed to gamma radiation from an atomic bomb source. Radiology 62: 409–415, 1954.
9. BROWN, D. G., CROSS, F. H., and GRAMLY, W. A. Effect of ^{60}Co gamma radiation dose rate on 30-day mortality in swine. Amer. J. Vet. Res. (in press).
10. BOND, V. P., and ROBINSON, C. V. A mortality determinant in nonuniform exposures of mammal. Radiat. Res. (Suppl.) 7:265–275, 1967.
11. KALLMAN, R. F. The effect of dose-rate on mode of acute radiation death of C57Bo and BALB/c mice. Radiat. Res. 16:796–810, 1962.
12. LIGIE, L. C., HARRIS, M. D., IATSCH, R. E., and VAN HOOSER, E. N. An analysis of the $LD_{50/30}$ as related to radiation intensity. Radiat. Res. 12:349–356, 1960.



FIGURE 5.—The femurs, tibias, and fibulas from a swine which survived unilateral (left side) exposure, 550 rads, to bomb radiation. The bones of the left leg are shorter and smaller in diameter. (This difference was apparently due to the higher dose to the left side. The animal survived 9.4 years postirradiation.)

13. NEAL, F. E. Variation of acute mortality with dose-rate in mice exposed to single doses of whole-body X-radiation. Int. J. Radiat. Biol. 2:295–300, 1960.
14. PAGE, NORBERT P. The effect of dose-protraction on radiation lethality of large animals. In *Dose Rate in Mammalian Radiation Biology*. Eds. D. G. Brown, R. G. Cragle and T. R. Noonan, Conf–680410, Clearinghouse for Federal Scientific and Technical Information, Springfield, Va., 22151, pp. 12.1–12.23, 1968.
15. HANKS, G. E., PAGE, N. P., AINSWORTH, E. J., LEONG, G. F., MENKES, C. K., and ALPEN, E. L. Acute mortality and recovery studies in sheep irradiated with cobalt-60 gamma rays or 1 Mvp X-rays. Radiat. Res. 27:397–405, 1966.
16. BROWN, D. G., GRAMLY, W. A., and CROSS, F. H. Response of 3 breeds of swine to whole-body cobalt-60 gamma radiation in daily doses of Roentgens. Amer. J. Vet. Res. 25:1347–1353, 1964.
17. KUHN, U. S. G., III., KYNER, R., and BROWN, D. G. Fractionated daily irradiation of swine. University of Tennessee-AEC Agricultural Research Laboratory Semiannual Progress Report ORO–169, 34 (Jan. 1–June 30, 1957).
18. BOND, V. P., FLIEDNER, T. M., and ARCHAMBEAU, J. O. Mammalian radiation lethality, a disturbance in cellular kinetics. AIBS Monograph, Academic Press, New York, 1965.
19. GRAHN, D. Genetic variation in the response of mice to total-body x-irradiation—The weight response of six inbred strains. J. Exp. Zool. 125:39, 1954.
20. KOHN, H. I., and KALLMAN, R. F. Influence of

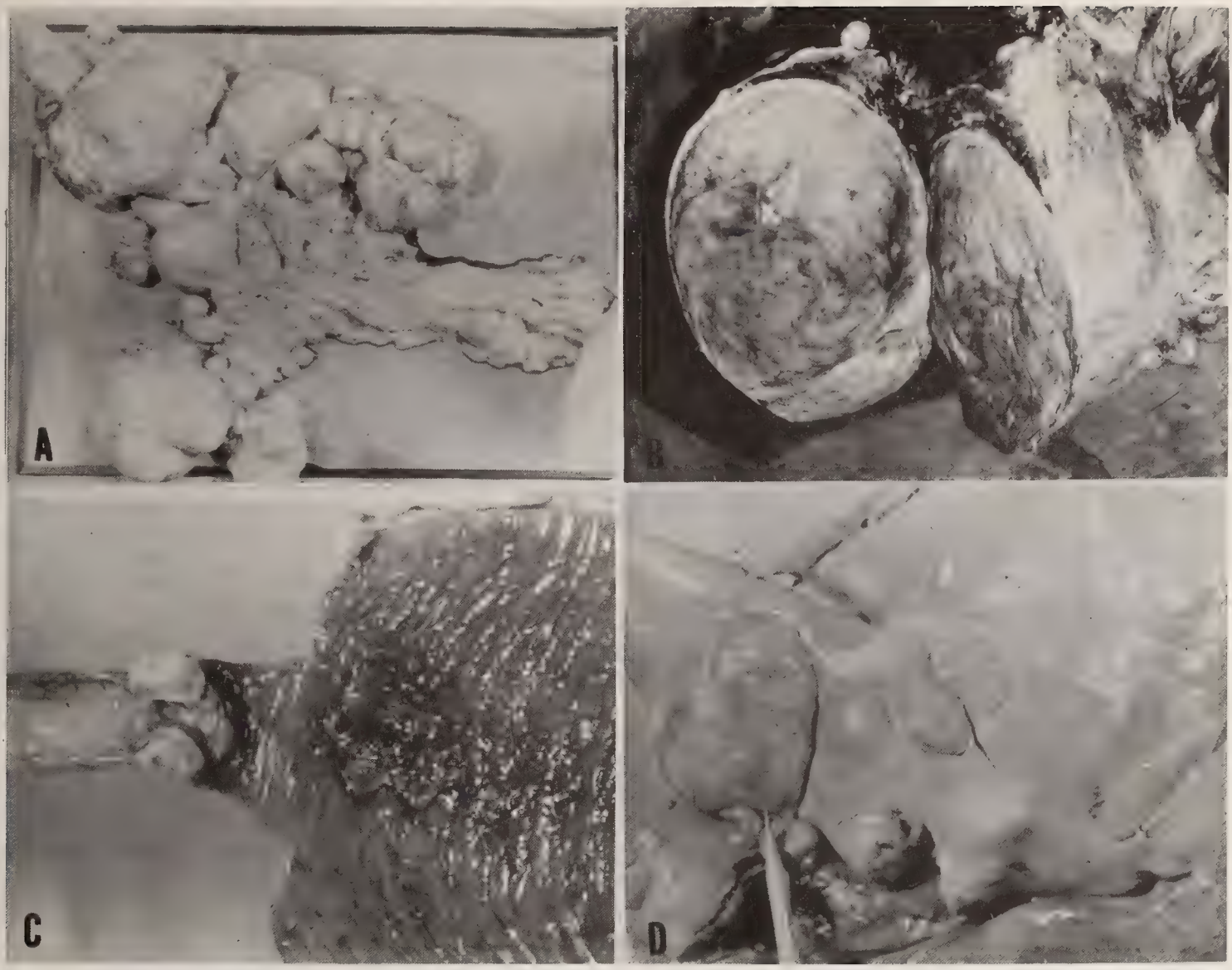


FIGURE 6.—The principal tumors which occurred at a relatively high frequency in irradiated swine. A. Leiomyas of the broad ligaments and uterus from a sow which survived 7.4 years after exposure to 250 rads of bomb radiation and a second exposure of 350 rads of X-rays 4 months after the initial exposure. B. Cross section of a uterine tumor (24 kg) from a sow which survived 8 years after exposure to 380 rads of bomb radiation. C. Adeno-carcinoma of the small intestine of a barrow which survived 4.9 years after exposure to 535 rads of bomb radiation. D. Hepatomas in a barrow which survived 6.4 years after exposure to 535 rads of bomb radiation.

strain on acute X-ray lethality in the mouse—LD_{50} in death rate studies. Radiat. Res. 5:309, 1956.

21. KOHN, H. I., and KALLMAN, R. F. The influence of strain on acute X-ray lethality in the mouse—Recovery rate studies. Radiat. Res. 6:329, 1957.
22. CASE, MARTIN T., and SIMON, J. Whole-body gamma irradiation of newborn pigs: The $LD_{50/30}$. Amer. J. Vet. Res. 31:113–115, 1970.
23. BROWN, DANIEL G., and JOHNSON, DONALD F. Clinical and gross pathological findings in swine relative to late effects of mixed gamma-neutron and X-irradiation. Radiat. Res. 44:498–511, 1970.
24. SHIVELY, J. N., ANDREWS, H. L., WARNER, A. R., MILLER, H. P., JURTZ, H. J., and WOODWARD, K. T. X-ray exposure of swine previously exposed to a nuclear detonation. Amer. J. Vet. Res. 25:1128–1133, 1964.
25. BROWN, D. G., and JOHNSON, D. F. Diseases of aged swine. J. Amer. Vet. Med. Assoc. 157:1914–1918, 1970.
26. TRUM, B. F., SHIVELY, J. N., KUHN, U. S. G. III, and CARLL, W. T. Radiation injury and recovery in swine. Radiat. Res. 11:326–342, 1959.
27. HALEY, T. J., MCCULLOH, E. F., MCCORMICK, W. G., TRUM, B. F., and RUST, J. H. Response of the burro

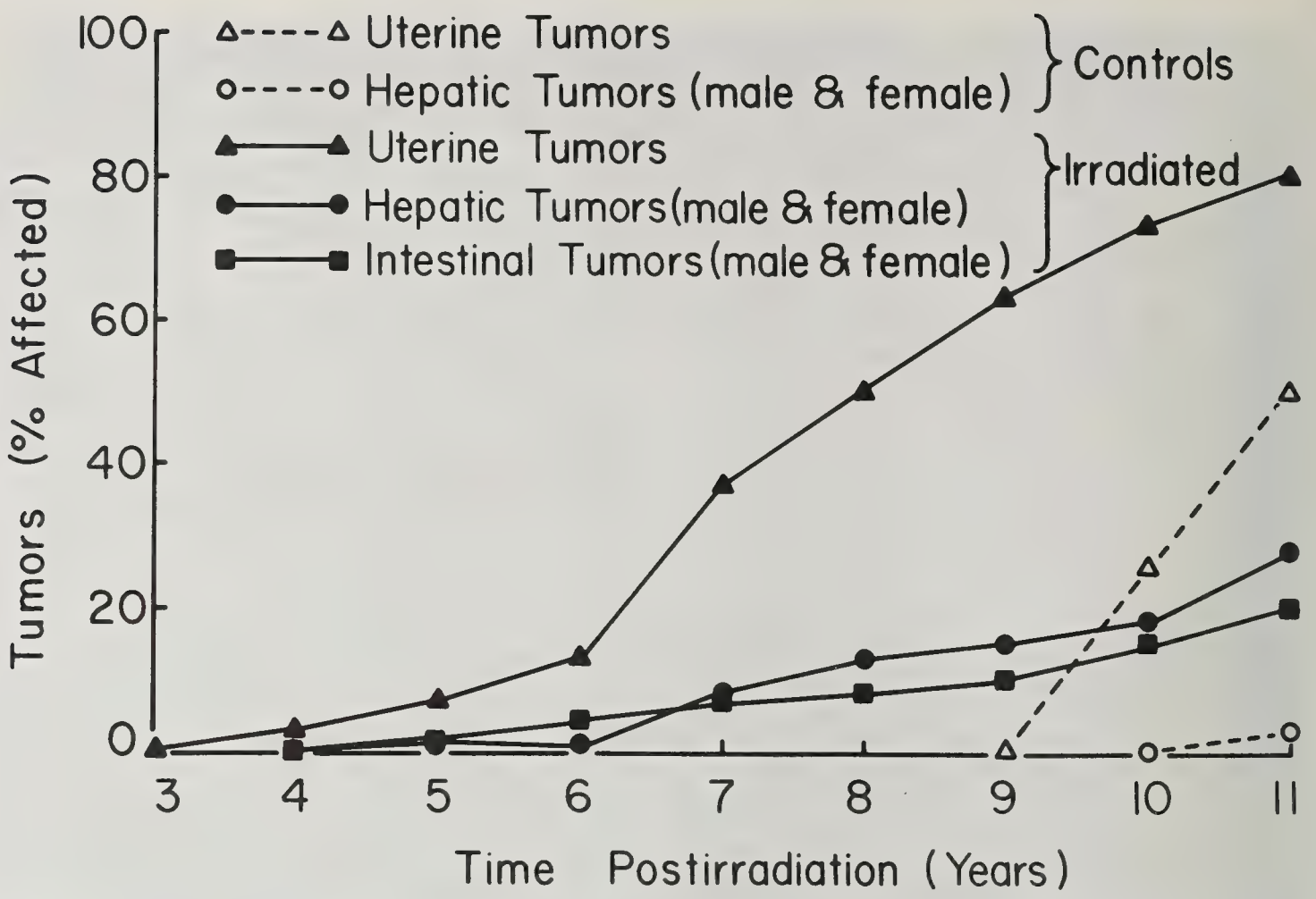


FIGURE 7.—The cumulative incidence of uterine, hepatic, and intestinal tumors in swine exposed to mixed gamma-neutron (bomb) and X-radiation and controls. Dose range: 15 to 850 rads.

to 100 R fractional whole-body gamma ray irradiation. Amer. J. Physiol. 180:403–407, 1955.

28. RUST, J. H., TRUM, B. F., LANE, J. J., KUHN, U. S. G. III, PAYSINGER, J. R., and HALEY, T. J. Effects of 50 roentgens and 25 roentgens fractional daily total-body γ-irradiation in burros. Radiat. Res. 2:475–482, 1955.

DISCUSSION

JOHN SESMENIC, University of Michigan: Did you or the pathologist speculate on a reason for the high incidence of uterine tumors and those of the broad ligaments? Is it just that that happened to be the tissue in line with where the radiation was coming into or through the animal or is there a more involved explanation?

DR. BROWN: Uniformity of dose is an important factor to consider. In this case the swine were positioned for unilateral exposure so that the dose-in-air would be equal from the anterior to posterior of the animals. We do not believe that the uterus and broad ligaments received doses larger than any other organs or tissues of equal depth in the swine. The data indicate that these tumors occurred earlier in the swine because of irradiation. Although the highest doses may have been sufficient to cause induction of tumors, we have doubts about this because of the incidence of tumors in the aged control swine.

H. A. RAGAN, Battelle Pacific Northwest Labs: Also in reference to these uterine tumors, I'd like to preface my question with a remark. We see near a 50 per cent incidence of uterine tumors in our miniature swine and this is not dose related. These tumors occur between six and eleven years of age. I'm wondering whether you know of any surveys of the incidence of uterine tumors in the swine population in gen-

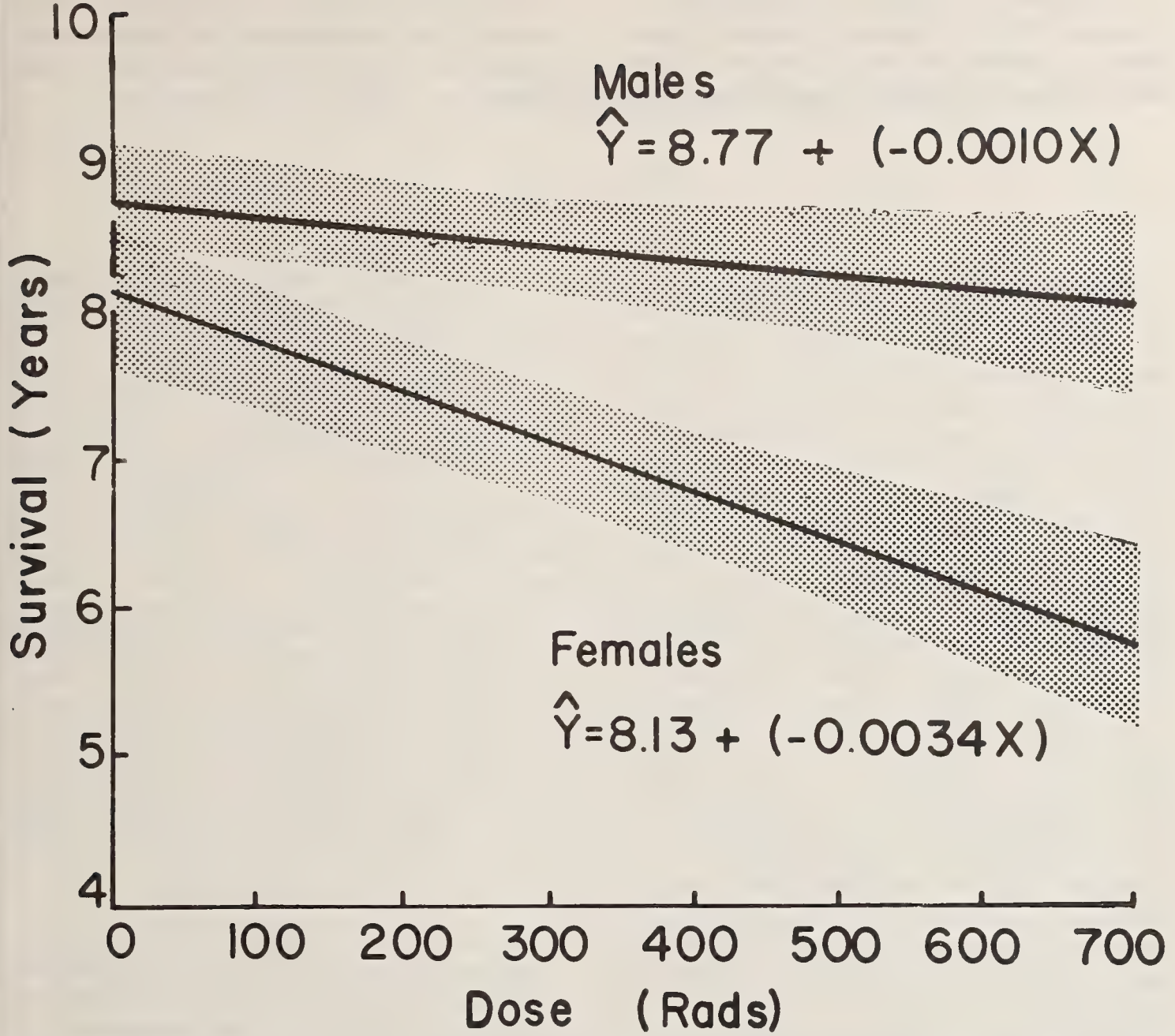


FIGURE 8.—Regression of postirradiation survival time on dose, with 68% confidence bands about the regression lines, for male and female swine exposed to mixed gamma-neutron (bomb) and X-radiation. (Life shortening for the total population was approximately 3% per 100 rads.)

eral? I realize they usually don't live this long, but I've been unable to find any.

DR. BROWN: To my knowledge there are two aged swine herds in the U. S. which have been used to study the normal aging process. One is at Iowa State University. This herd should now be about 10 years old. At nine years of age no tumors had been found in these swine. The other herd is in New Jersey and was used by the Veterinary School of the University of Pennsylvania for studies with atherosclerosis. Some of the animals which they used, and had an opportunity to perform autopsies on, apparently lived to be about fifteen years old. The incidence of tumors in these swine was approximately 3 per cent for the hepatoma and 3 per cent for the intestinal tumors, however, the intestinal tumors which they found in these swine were in the colon rather than in the small intestine.

MCCLELLAN G. BLAIR, ARCO Nuclear Co.: (Inaudible)

DR. BROWN: To what do I contribute the difference in RBE for swine with respect to man? I have no answer for that. As far as I know, there has been no logical reason why neutrons are less effective in large animals than in small animals. This was discussed in a neutron symposium approximately two years ago at Oak Ridge.

EXTRACORPOREAL IRRADIATION OF BLOOD AND LYMPH

D. D. Joel and E. P. Cronkite*

Methods have been developed for continuous or intermittent extracorporeal irradiation of circulating blood (ECIB) and thoracic duct lymph (ECIL) in calves and goats. ECIB and ECIL both induce a marked and sustained lymphopenia as well as a decrease in the thoracic duct lymphocyte output and a depletion of an easily mobilizable pool of lymphocytes from lymphoreticular organs. A significant pool of sessile lymphocytes is unaffected by these procedures.

The functional aspects of the mobilizable pool of cells have been tested by challenging ECIB or ECIL treated animals with tetanus toxoid and/or orthotopic allografts. The primary and secondary antibody responses to tetanus toxoid were only slightly repressed by pretreatment with either repetitive ECIB or continuous ECIL. Repetitive ECIB given prior to grafting will prolong skin allograft acceptance time 2 to 3 days. Further graft survival was obtained, however, when ECIB was continued following grafting. With continuous ECIL skin allograft survival was dependent upon the location of the graft. As with skin grafts, kidney allograft survival time was longest in animals treated with ECIB both before and after transplantation.

INTRODUCTION**

Extracorporeal irradiation was apparently first conceived of as early as 1921,[1] however, it received little attention until the early 1960's. The development and use of extracorporeal irradiation as a method to deplete the body of lymphocytes for purposes of studying various aspects of cell physiology in normal and leukemic calves has been described in detail in a series of publications from the Brookhaven National Laboratory.[2–12] In this report we will briefly review the methods used, and their effect on lymphoid tissues and immune responses, particularly transplantation immunity.

The underlying principle of extracorporeal irradiation is to utilize the difference in susceptibility to injury by irradiation between circulating lymphocytes and other formed elements in blood or lymph, i.e., doses which are lethal to the vast majority of lymphocytes are relatively harmless to red blood cells, neutrophils and platelets. In practice, two experimental approaches have been developed. The first, extracorporeal irradiation of blood (ECIB), is accomplished by diverting a fraction of the cardiac output through a radiation field by means of a semipermanent carotid artery-jugular vein shunt composed of Teflon and Silastic[11] (Figure 1). During ECIB, cells within the blood accumulate a dose of radiation as they pass one or more times through the irradiator. The amount of radiation received by a cell during one circuit past the radiation source is referred to as the transit dose (transit doses ranging from 15 to 1,500 rads have been used in various studies). When a lethal dose is attained the cell is removed from the circulation by the reticuloendothelial system. Although ECIB can be given continuously or in short repetitive sessions, there is a limit on the total amount of ECIB which can be tolerated within a given period of time. This limit is determined by the accumulated dose to the red blood cells which, because of their relatively long intravascular life span, may make several transits through the radiation field.[4] In calves, red cells can accumulate up to 50,000 rads before hemolysis begins,[13] while in goats the tolerable dose is much lower i.e., about 10,000 rads. For this reason and other considerations pertaining to exchange of lymphocytes between various tissue and circulating pools, daily repetitive ECIB is preferred over continuous ECIB.

The second experimental approach which has been developed is extracorporeal irradiation of lymph (ECIL). In this system thoracic duct lymph is simply diverted through the radiation field using Teflon-Silastic shunts.[11] Because of the low hydrostatic pressure of efferent lymph,

* Medical Research Center, Brookhaven National Laboratory, Upton, New York.

** Work supported by the U.S. Atomic Energy Commission.

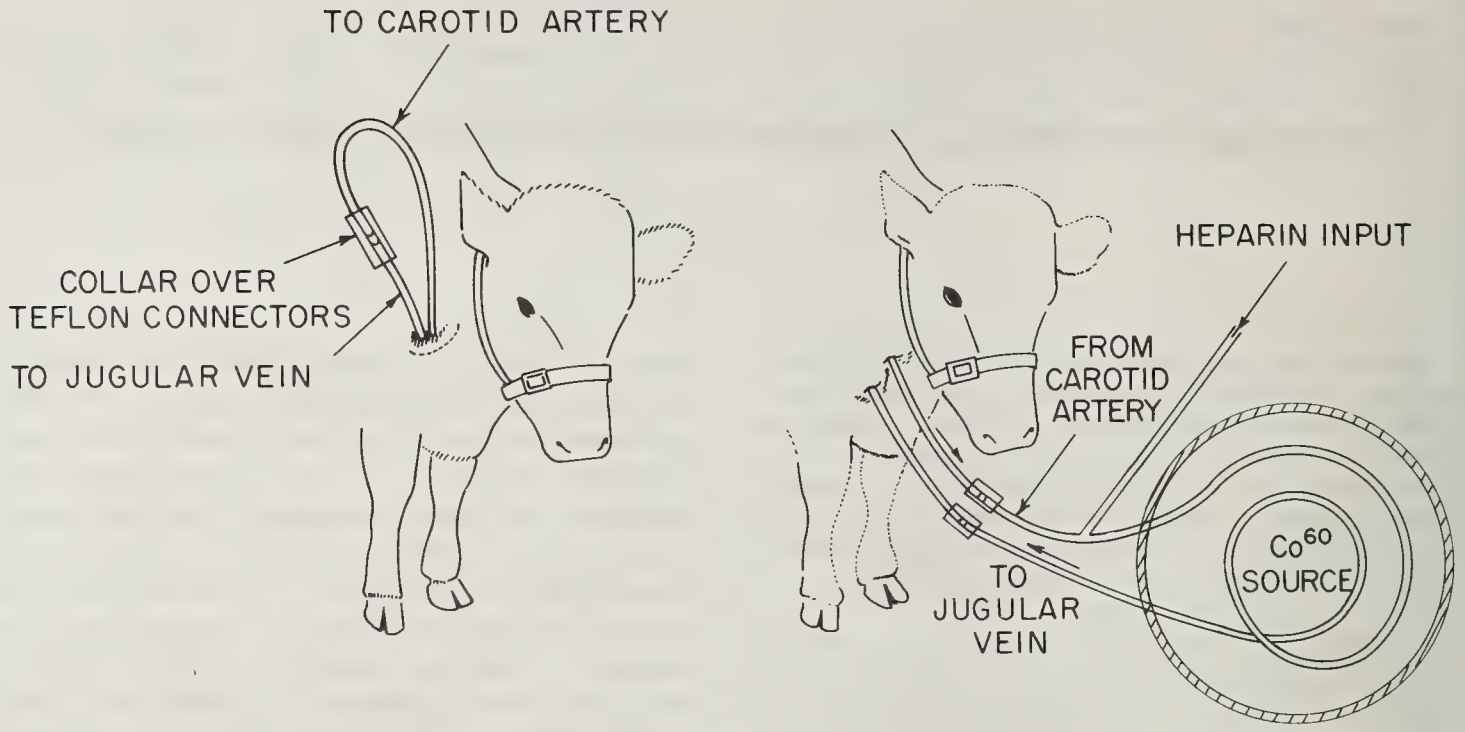


FIGURE 1.—Schematic representation of extracorporeal irradiation of blood (ECIB) in calves. (Reprinted from Sipe et al., 1965.)

a pumping system is required to propel lymph through the irradiator and back into the venous system. Thoracic duct lymph contains very few red blood cells thus ECIL can be given continuously for long periods of time (weeks to months) without complication. As with ECIB, the primary objective is to deplete the body of lymphocytes. One fundamental difference in the two methods is that, whereas ECIB destroys a certain fraction of all lymphocytes entering the blood irrespective or origin, ECIL destroys only those lymphocytes which either enter the thoracic through recirculation from blood to lymph or are produced in the regional lymph nodes.

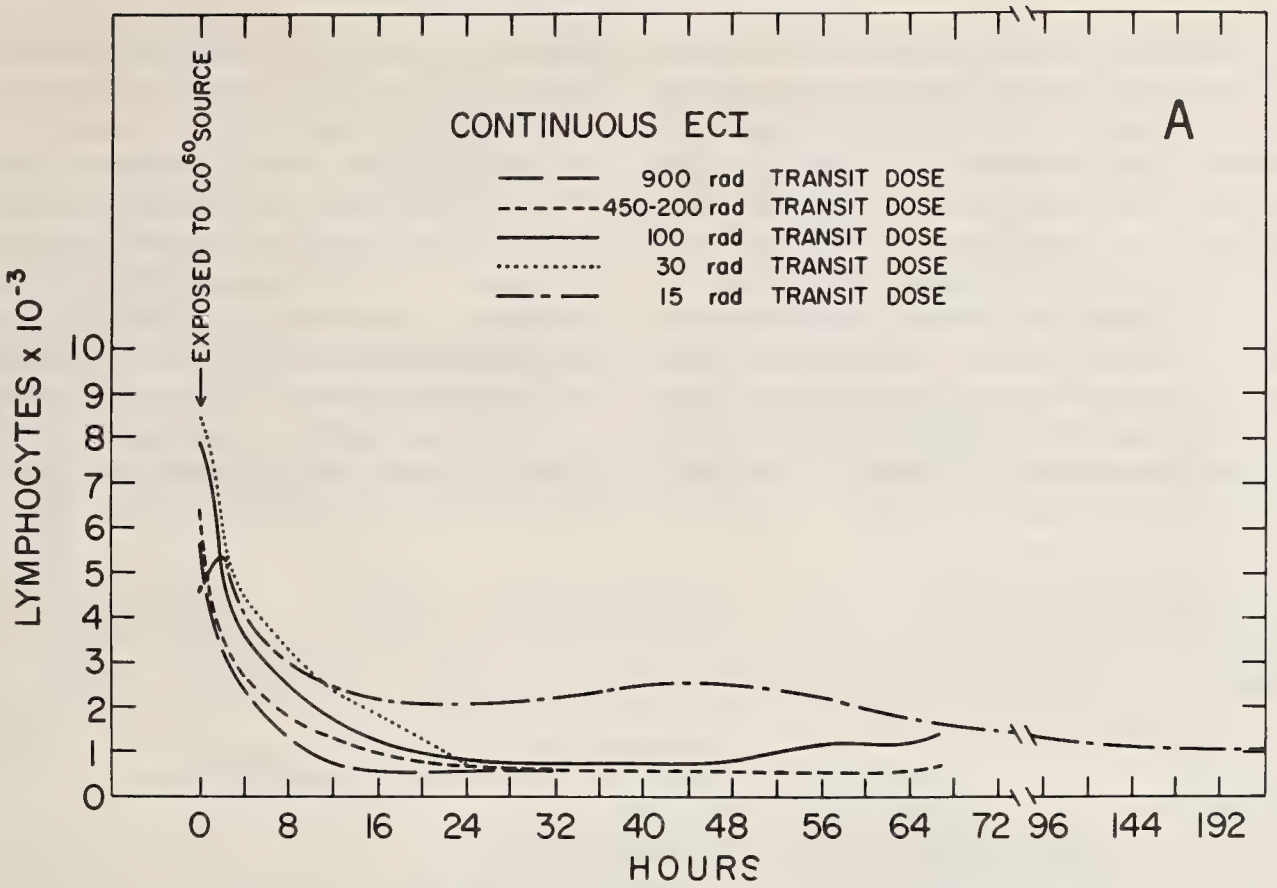
LYMPHOCYTE DEPLETION BY ECIB AND ECIL

Prolonged continuous ECIB or daily repetitive ECIB produces a marked lymphopenia (Figure 2a). Transit doses between 15 and 900 rads are effective, however, lymphopenia develops more rapidly with higher doses, i.e., above 200 rads. Daily repetitive ECIB also results in lymphopenia (Figure 2b) and is, in fact, more efficient than continuous ECIB in terms of the total radiation exposure to the blood. Increased efficiency with repetitive ECIB no doubt results from the establishment of new equilibria between tissue and blood pools of lymphocytes during intervals when no ECIB is given. Since lymphocytes have a short intravascular transit time,* thereby reducing the probability of making multiple passes through the irradiator, the most efficient schedule of ECIB should be daily repetitive sessions in which 5–10 blood volumes are irradiated at a transit dose sufficiently high to kill the majority of lymphocytes on one passage through the radiation field.

ECIB also results in a substantial reduction in the output of lymphocytes in the thoracic duct.[6] This finding provides direct confirmation that lymphocytes circulate from blood to lymph [14] and that the lymphocyte concentration in efferent lymph is directly dependent upon the lymphocyte concentration in the blood.

Morphological classification of blood lymphocytes into large and small categories suggested that ECIB had a greater effect on small lympho-

* Based on the number of lymphocytes emerging in thoracic duct lymph and thymic vein blood alone, the mean intravascular time of lymphocytes would be less than 3 hours in calves. Transfusion of labeled lymphocytes indicates the minimum transit time to be between 15 and 30 minutes.



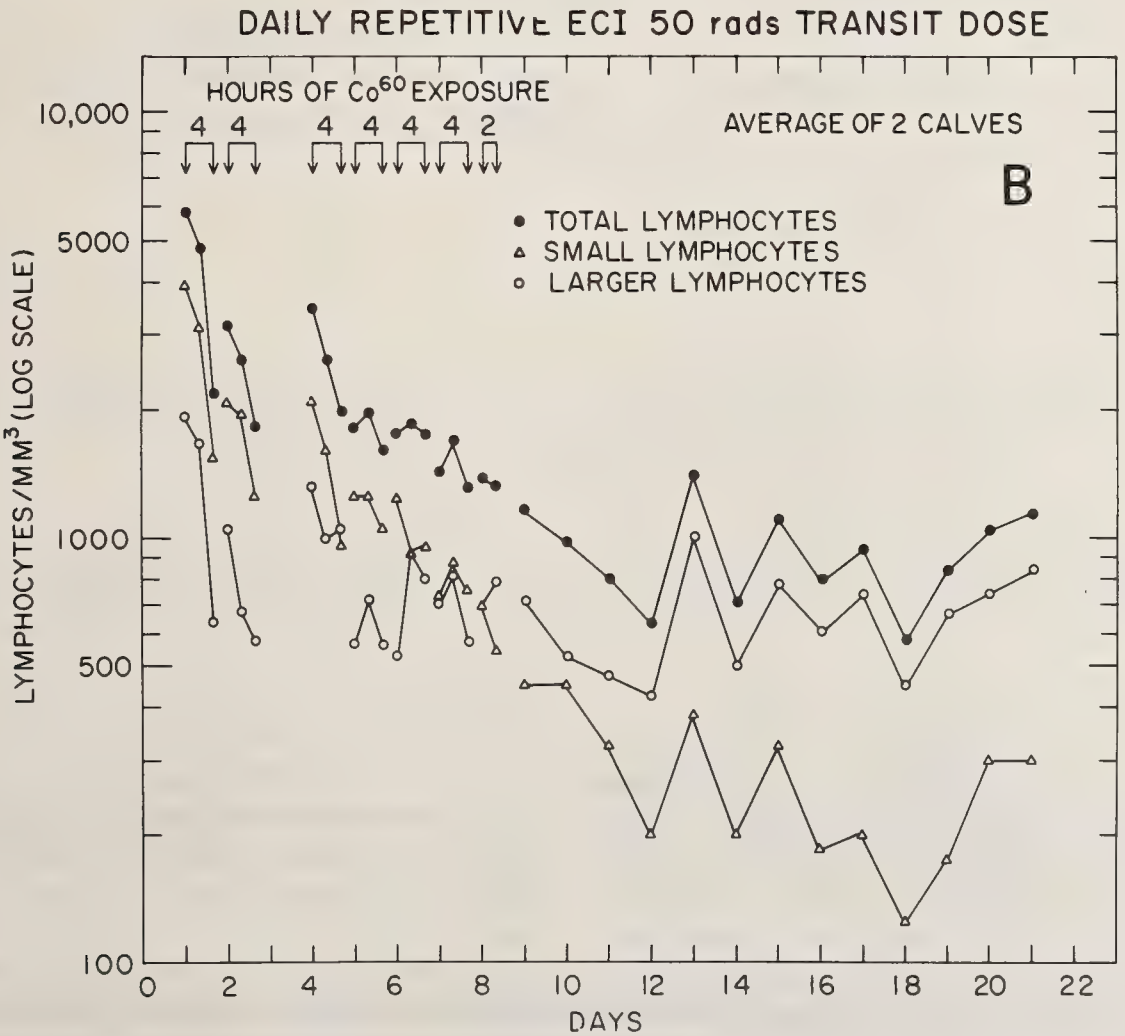


FIGURE 2.—a. Total blood lymphocyte count as a function of transit dose and time after commencement of ECIB. 900 rads represents 10 calves: 450–200 rads represent 10 calves; 100 rads represents 2 calves; 30 rads represents 3 calves; 15 rads represents 5 calves. (Reprinted from Sipe et al., 1965.) b. The influence of repetitive daily ECIB on the blood lymphocyte counts plotted on semilog paper. (Reprinted from Sipe et al., 1965.)

cytes (Figure 2b) as compared to large lymphocytes. Direct verification of this effect was established in size distribution studies on suspensions of thoracic duct lymphocytes.[15] In untreated calves the volume distribution of thoracic duct lymphocytes is a continuum between 120 and 1,200 μ^3 with a mode between 240 and 288 μ^3 (Figure 3). These data, when plotted on logarithymic probability paper, indicated the presence of two populations of lymphocytes: (1) a small population with a median cell volume of 250 μ^3 and constituting 90–95% of the total, and (2) a large population with a median cell volume of 648 μ^3. Lymphocytopenia induced by either ECIB or ECIL markedly alters the size distribution (Figure 3) due principally to a loss of cells within the small lymphocyte category.

Continuous ECIL also results in a marked decrease in the blood lymphocyte count and cellular output in the thoracic duct as illustrated in Figure 4, where large and small lymphocytes are plotted separately. Lymphocyte depletion of both blood and lymph did not occur according to a single exponential function but rather followed a curve with at least two components. This is shown in Figure 5 for the thoracic duct

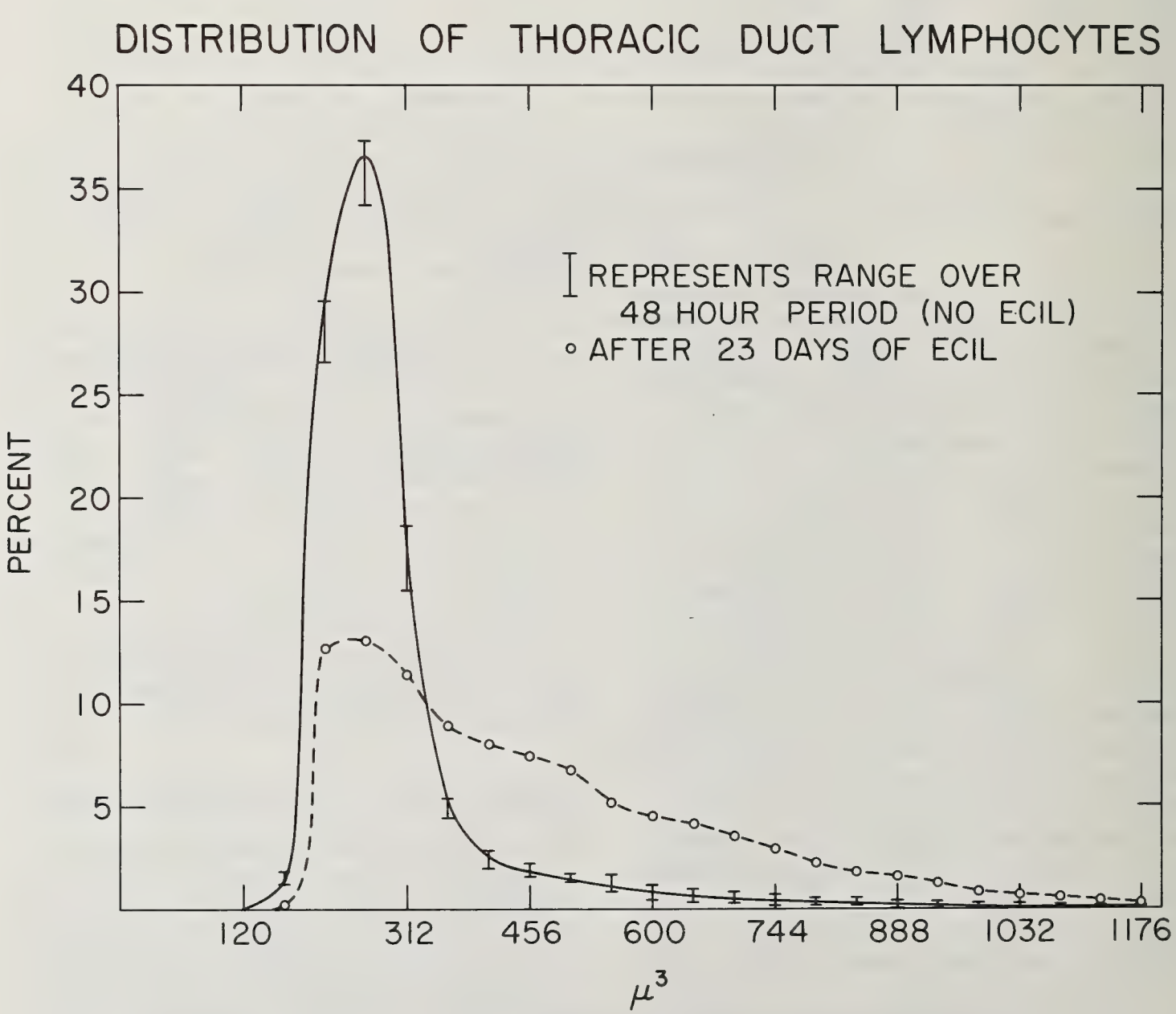


FIGURE 3.—Distribution curve of thoracic duct lymphocytes from calves which received no treatment (——) and from a calf which had 23 days of continuous extracorporeal irradiation of lymph (ECIL) (o——o).

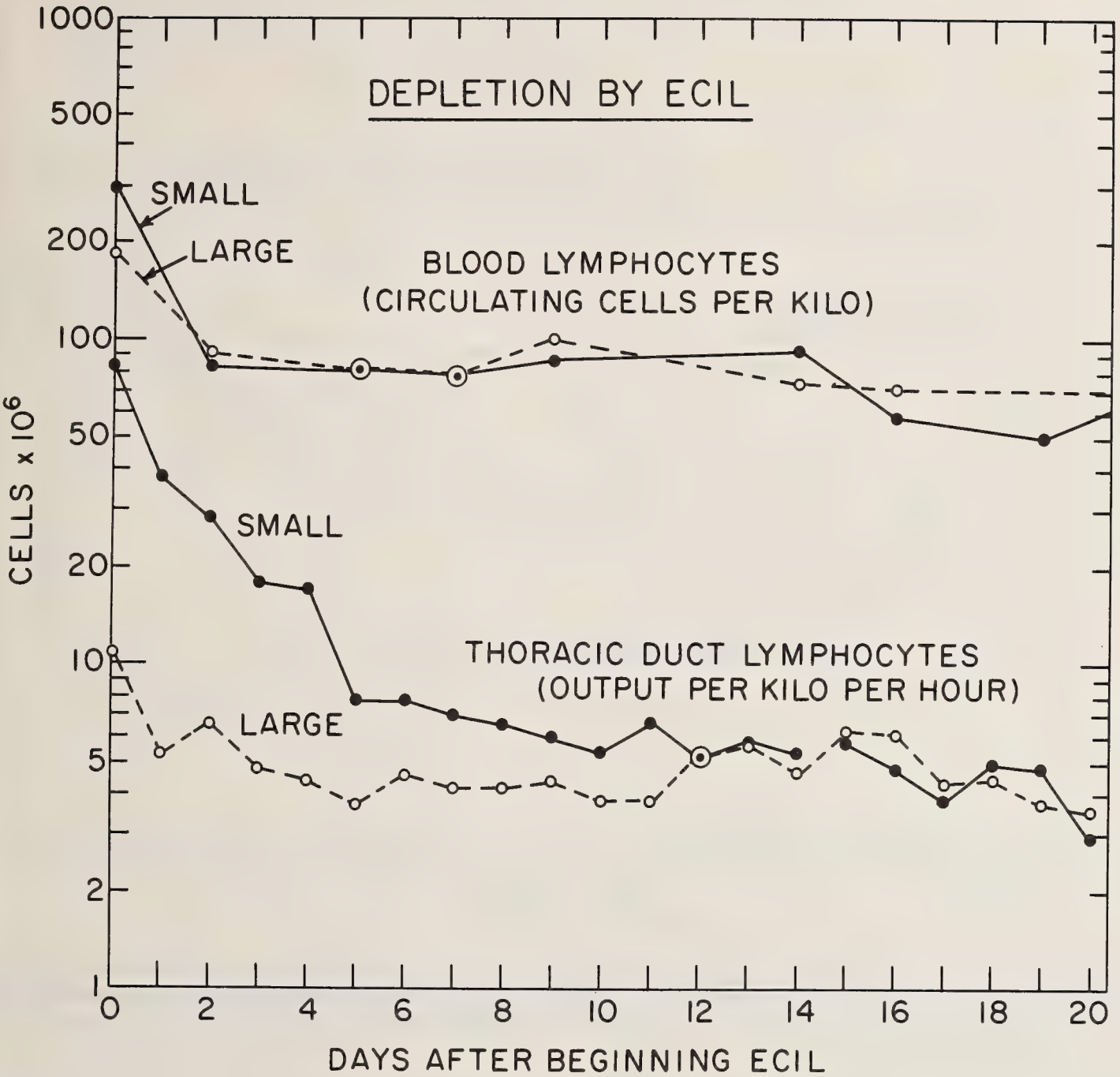


FIGURE 4.—The effect of continuous ECIL on blood lymphocytes and thoracic duct cellular output. Lymphocytes are classified as large (●——●) or small (○.....○) according to cell diameter in blood smears and electronically Coulter Model F) in thoracic duct lymph.

output of small lymphocytes. The first component, with a halving time of 1.2 days, corresponds to the elimination of an easily mobilizable pool of lymphocytes and the second component (halving time of approximately 30 days) corresponds to a more sessile mass of lymphocytes. Calculations based on these data indicate the size of the easily mobilizable pool to be approximately 10 times the number of circulating blood lymphocytes, or 3–5 $\times$ 10^9 lymphocytes/kg body weight.[16]

It is well known that a fraction of blood lymphocytes cycles from blood to lymphoreticular tissues and back again.[14] Therefore, the destruction of circulating lymphocytes by ECIB or ECIL results lymphocyte depletion of lymphoreticular organs.[8,17] The relative lymphocyte depletion of various areas of spleen and lymph

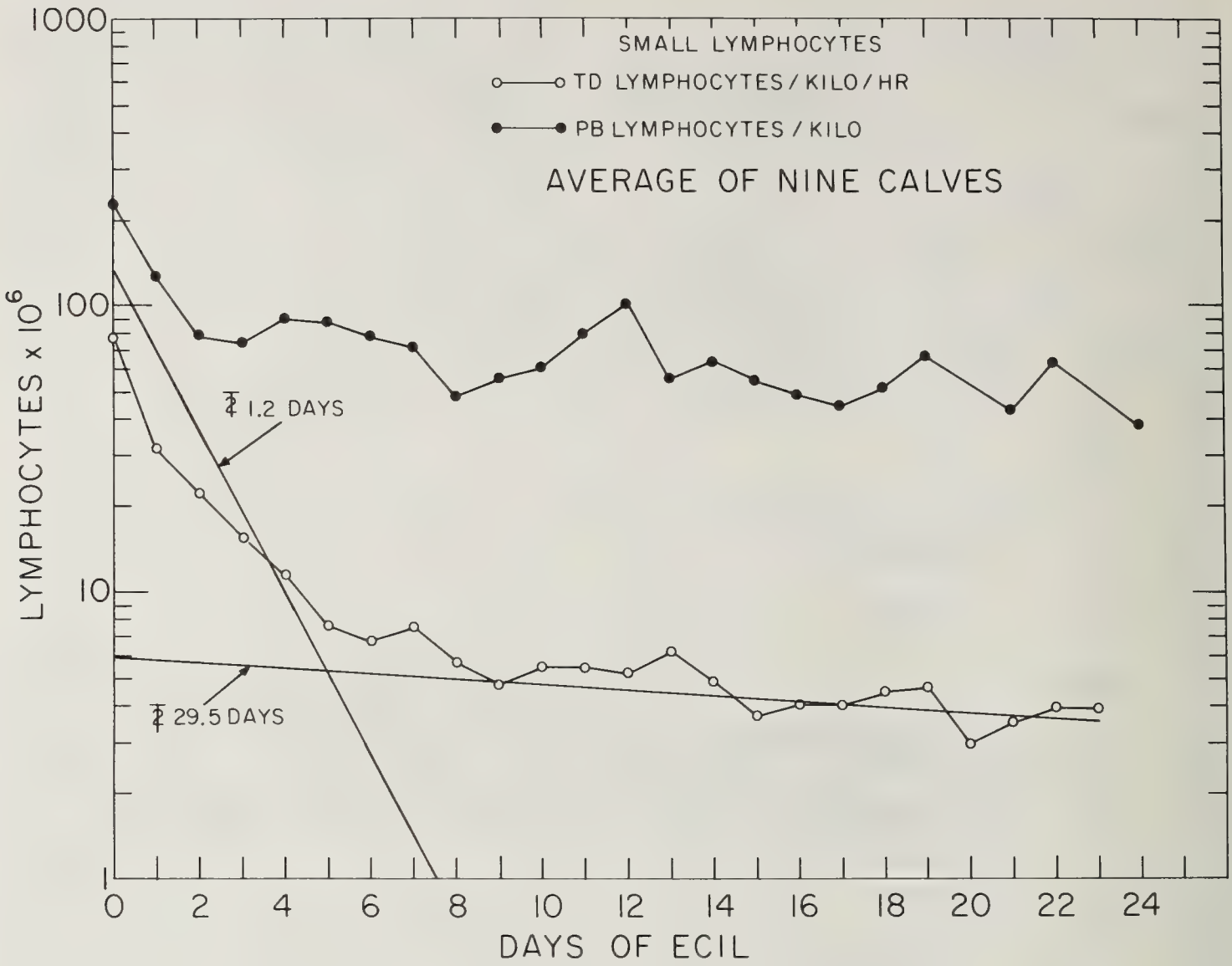


FIGURE 5.—The effect of continuous ECIL on small lymphocytes in the peripheral blood (PB) (●——●) and thoracic duct (TD) lymph (O.....O). The decrease in thoracic duct output is represented by two components: the first with a halving time of 1.2 days; the second with a halving time of 29.5 days. (Reprinted from Cronkite et al., 1968.)

nodes as a function of time after onset of continuous ECIB is shown in Figure 6. As with blood and lymph, depletion of lymphoreticular organs follows an exponential with two components. Best estimates of the relative size of the easily mobilizable pool are: lymph node medulla $< 10\%$, lymph node cortex 18%, splenic red pulp 37%, splenic dense white pulp 55%, and splenic loose white pulp 60%. Germinal centers remain intact.

Recovery from lymphocyte depletion induced by ECIB or ECIL is prolonged.[5] Depending upon the degree of depletion, blood lymphocyte counts may remain below pre-irradiation levels for at least 6 months. This is mainly due to a lag in recovery of small lymphocytes as large lymphocyte levels return to normal in about 3 weeks.

Collectively, these studies indicate that ECIB and ECIL are effective in depleting the body of an easily mobilizable pool of lymphocytes which circulate from blood to lymphoreticular organs and back again. This pool is composed principally of small, non-dividing, long-lived lymphocytes which following depletion are replaced very slowly. Thymectomy and lymphocyte labeling experiments in rodents suggest that the recirculating pool of lymphocytes is principally

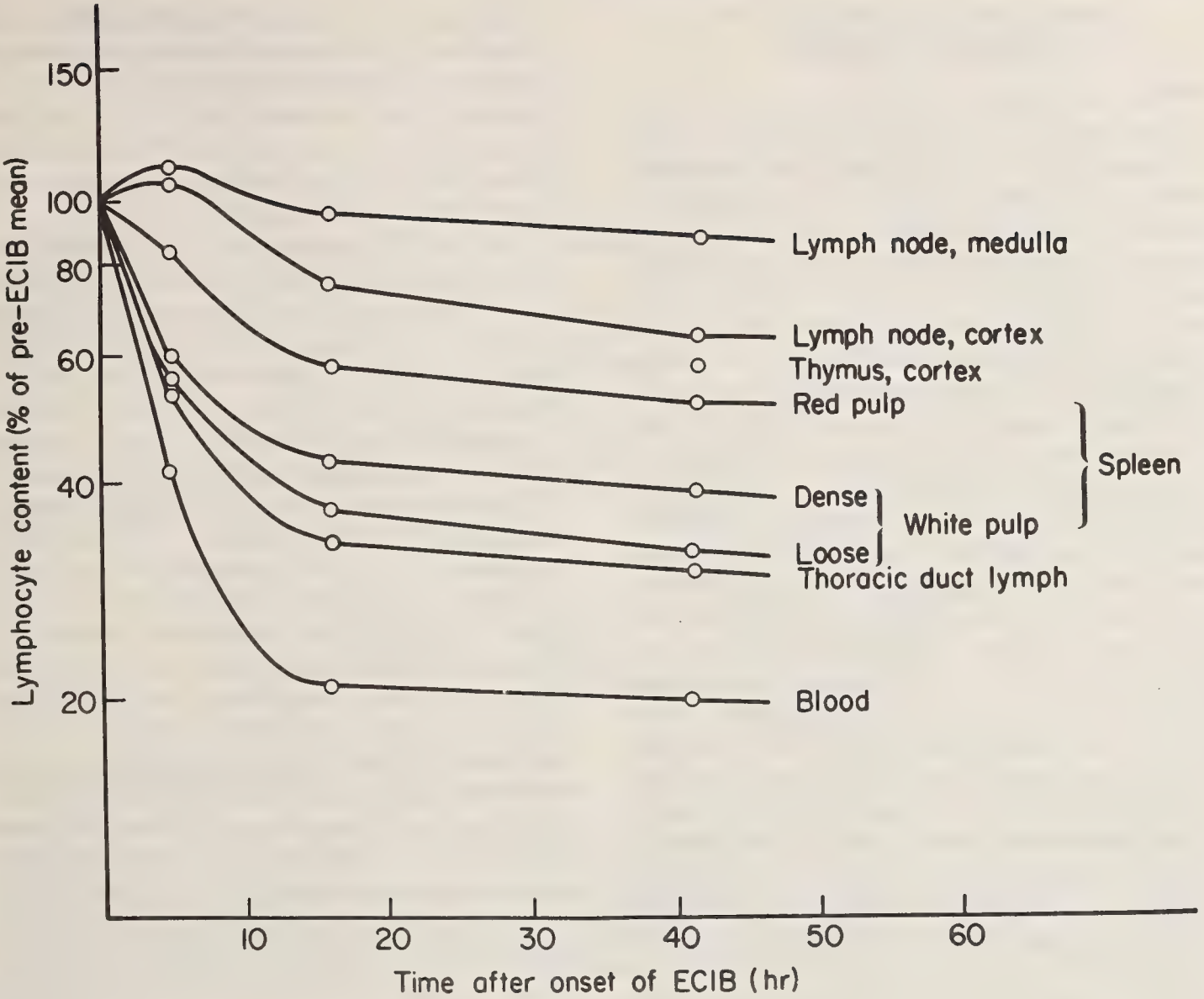


FIGURE 6.—Lymphocyte depletion in various areas of lymphoreticular organs, in the thoracic duct lymph and in the blood of the calf as a function of time after onset of continuous ECIB. (Reprinted from Ruchi et al., 1970.)

of thymic origin. It is of interest that recent studies provide evidence that, in the calf, the thymus issues into the blood a sufficient number of lymphocytes to totally replace the easily mobilizable pool in less than 5 days.[18] It thus becomes quite evident that the vast majority of thymocytes in calves never enter the recirculating pool. Large lymphocytes make up a small fraction of the mobilizable pool as evidenced by a decrease in these cells in the blood and thoracic duct lymph during ECIB or ECIL. In contrast to small lymphocytes recovery is relatively rapid.

The size of the sessile pool of lymphocytes is difficult to measure for several reasons. (1) Estimates based on thoracic duct outputs during ECIL must take into account the input from formation of new cells and the contribution by afferent lymphatics. Neither of these are known. (2) The relative size of the sessile pool in lymphoreticular organs can be estimated by planimetry, however, this does not include lymphocytic infiltrates at sites other than the lymphoreticular organs. It seems probable that the sessile pool is considerably larger than the easily mobilizable pool particularly in lymph nodes.

EFFECT OF ECIB AND ECIL ON IMMUNE RESPONSES

Antitoxin Response

The effects of extensive repetitive ECIB and continuous ECIL on the primary and secondary antitoxin responses in calves to stimulation by tetanus toxoid have been published.[19,20] Although the appearance of detectable serum antibody was delayed 5 to 10 days and peak titers were slightly reduced, all calves gave nearly normal primary responses despite the severe depletion of lymphocytes in the circulating blood and thoracic duct lymph. This is in contrast to the findings of McGregor and Gowans [21] in rats depleted of lymphocytes by drainage of the thoracic duct for 5 days. It should be emphasized that in the rat experiments fluid toxoid was given by intraperitoneal injection, whereas the calves were immunized with a subcutaneous injection of either aluminum phosphate absorbed toxoid [19] or fluid toxoid.[20] Antigenic stimulation by intraperitoneal injection relies heavily on splenic participation as compared to regional lymph nodes. As shown above, the spleen was depleted to a much greater degree by ECIB than were lymph nodes. If the same is true for thoracic duct drainage in the rat one might anticipate greater immunosuppression to systemic immunization. The effect of ECIB and/or ECIL on systemically administered antigens has not been tested. Antigenic dose per kg body weight, which was much higher in the rat experiments, may also be important particularly in lymphocyte depleted animals. Extensive repetitive ECIB also failed to significantly repress secondary responses to fluid tetanus toxoid.

Skin Allografts

Repetitive ECIB given prior to skin grafting prolonged the graft acceptance time by 2 or 3 days and changed the normal violent skin allograft rejection to more chronic, milder reaction.[22] As a consequence of the severe lymphopenia, cellular infiltration into the graft bed was markedly reduced. When repetitive ECIB was continued after grafting, the acceptance time was further prolonged. Of special interest is the observation that ECIB was most effective in prolonging skin allograft survival when used in combination with small doses of azothioprine (Imuran[R]) which themselves had no effect on rejection. This suggests that ECIB and immunosuppressive therapy may be synergistic. Thymectomy combined with ECIB was no more effective than ECIB alone.[23]

The effect of continuous ECIL on skin allograft rejection is somewhat different. Prolonged ECIL (10–22 days) prior to grafting resulted in a 5 to 8 day prolongation of graft survival which was slightly better than that achieved by pregraft ECIB. When ECIL was continued after grafting, graft survival became dependent upon the anatomical location of the grafts.[24] The probable explanation for these findings are schematically shown in Figure 7. Following the transplant of a histo-incompatible skin graft, committed lymphocytes ("killer cells") are produced in the regional lymph node, presumably through antigenic stimulation and cell division. These cells enter the blood via the efferent lymphatics and return to the graft site where they effect rejection. It can be seen that, whereas ECIB will destroy only a fraction of cells entering from the efferent lymph, ECIL will affect all such cells provided the skin graft is placed within the drainage bed of the efferent lymph being irradiated. In the case of ECIL, all efferent lymph from the posterior part of the body enters the thoracic duct and passes through the irradiator. Thus when ECIL is continued following grafting, posteriorly placed skin grafts will survive for the duration of ECIL while skin grafts on other parts of the body (anterior grafts) will be rejected, provided that: (1) lymphatico-venous communications other than the thoracic duct are absent; and (2) anterior and posterior grafts are from two unrelated donors. The results are shown in Table I. It is of interest that postgraft ECIL also extended the survival of anterior grafts, suggesting that committed cells recirculate through the thoracic duct and are thus destroyed by ECIL. These experiments demonstrate that: (1) viable cells emerging from regional lymph nodes are essential for skin allograft rejection and these cells are susceptible to injury by irradiation; and (2) entry of committed lymphocytes into the blood is obligatory via the efferent

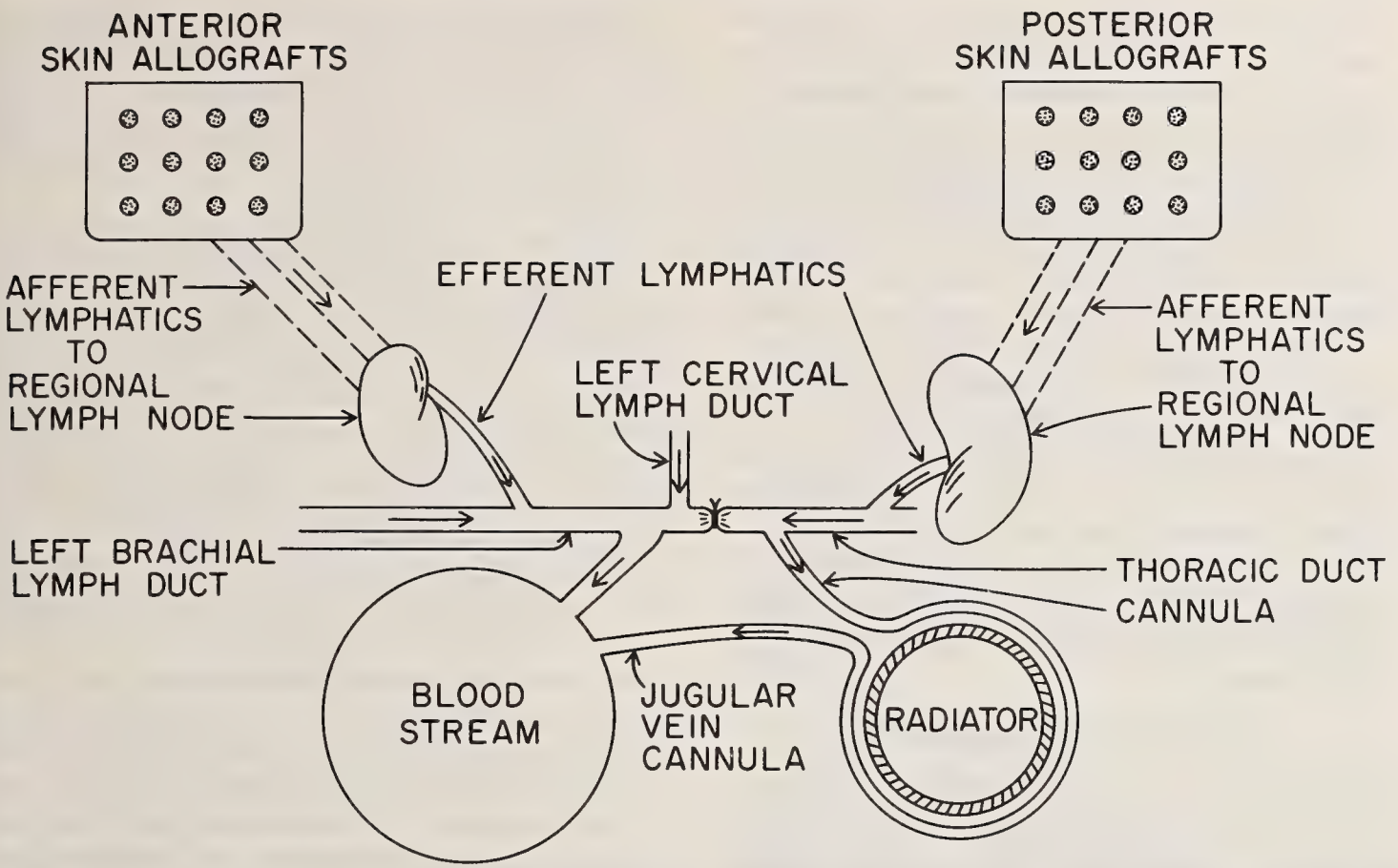


FIGURE 7.—This diagrammatic sketch shows the probable anatomical pathways of committed lymphocytes from the lymph nodes draining anterior and posterior skin allografts. Cannulation of the thoracic duct leaves the flow of lymph from the left brachial duct into the venous confluence uninterrupted. In the absence of any lymphaticovenuos communications, it is possible by the creation of a thoracic duct-venous shunt to irradiate all of the committed lymphocytes from the regional lymph nodes draining the site of posterior skin allografts. (Reprinted from Chanana et al., Transplantation 7:459, 1969.)

lymphatics, i.e., they do not enter the blood directly within the lymph node. Transit doses employed in this series of experiments varied between 350 and 1,800 rads.

TABLE I.—*Effect of Extracorporeal Irradiation of Thoracic Duct Lymph (ECIL) on the Survival of Skin Allografts in Calves.*

	Duration (Days) of ECIL		Day of Graft Rejection	
Calf	Pregraft	Postgraft	Anterior	Posterior
12 control	0	0	8–11	—
203	0	0	10	10
186	22	0	17	—
239	10	0	18	18
200	7	27	22	39
202	7	61	27	61*
206	0	25	13	28
257	0	21	—	26
296	0	28	—	37
295	0	11	16	—

* Died of acute septicemia on day 61. Grafts were intact at time of death. (Reprinted from Joel et al., 1967)

Renal Allografts

More recently we have been interested in the effect of ECIB on renal allograft survival. Because of cost, availability and size, goats were used instead of calves. These studies are designed to: (1) determine the effectiveness of ECIB alone in prolonging renal allograft rejection, (2) determine the most effective schedules of ECIB, and (3) study the combination of ECIB and standard immunosuppressive drug therapy. These investigations were prompted by the fact that, even though ECIB is already being used in a number of hospitals as an adjunct to drug induced immunosuppression, no controlled animal experiments have been completed.

To date essentially three schedules of ECIB have been studied.[25] The first schedule included

only repetitive pregraft ECIB at a relatively high transit dose (300–400 rads). Due to increased susceptibility of goat red blood cells to hemolysis by irradiation, relatively little ECIB could be given at these dose ranges. Consequently, a second schedule of repetitive pregraft ECIB was employed using a low transit dose (45–55 rads) but nearly doubling the total number of blood volumes irradiated. A third schedule included both pre- and post-graft repetitive ECIB using the low transit dose. The preliminary results are shown in Table II. These findings indicate that: (1) the total number of blood volumes irradiated prior to grafting may be an important aspect of immunosuppression by ECIB; and (2) without question pre- and post-graft ECIB is superior to pre-graft ECIB only. This was also true in experiments with skin allografts.

CONCLUSIONS/SUMMARY

ECIB and ECIL are effective methods for inducing marked lymphocytopenia and depleting the body of an easily mobilizable pool of lymphocytes which circulate from blood to lymphoreticular organs and back again. This mass of rapidly recirculating cells is composed principally of small nondividing lymphocytes which, when depleted, is replenished at a very slow rate. A fraction of the recirculating pool removed by extracorporeal irradiation is composed large lymphocytes. In contrast to small lymphocytes, recovery following depletion is relatively rapid.

The sessile pool of lymphocytes in lymphoreticular organs is much less affected by extracorporeal irradiation because of the apparent slow exchange of cells between blood and tissue compartments. Kinetic studies[26] indicate that the sessile pool also has a slow replacement rate, and from the depletion experiments discussed above, it would appear that the total pool size is considerably larger than the easily mobilizable mass of lymphocytes.

TABLE II.—*Effect of Extracorporeal Irradiation of Blood (ECIB) on the Survival of Renal Allografts In Goats.*

ECIB Schedule	Number of Goats	Survival (Days)
Control	8	16.9
I	8	17.6
II	9	30.3
III	8	48.1

Schedule I = Repetitive pregraft ECIB at transit dose of α 330 rads; Schedule II = Repetitive pregraft ECIB at transit dose of α 50 rads; Schedule III = Repetitive pre- and postgraft ECIB at transit dose of α 50 rads.

The functional aspects of lymphocytes in the mobile versus sessile pools were tested by antigenic stimulation with tetanus toxoid, skin allograft and renal allograft rejection. ECIB and ECIL only slightly reduced primary and secondary responses to tetanus toxoid and moderately prolonged skin allograft survival. Therefore, it would appear that within the sessile pool are: (1) antigen-responsive lymphocytes capable of initiating primary immune responses, (2) sensitized cells capable of eliciting a typical secondary immune response, and (3) immunocompetent cells able to differentiate into cells capable of allograft destruction. Since all of these responses were somewhat altered by ECIB and ECIL, it can be assumed that the same cell types are present in the mobile pool. Therefore, immunosuppression to allografts would be most effective when extracorporeal irradiation is conducted before and after grafting. This was particularly evident in the prolongation of renal allografts by ECIB.

Further immunosuppression by extracorporeal irradiation could no doubt be achieved by "mobilization" of the sessile pool. Several compounds are known to induce an increase in the blood lymphocyte count including heparin[27] which is used to prevent clotting during ECIB or ECIL. Bordetella pertussis and polymethacrylic acid will increase (2 to 2.5 times) the blood lymphocyte count in animals depleted by ECIB. The total mass of cells "mobilized" by these agents in the depleted state is, however, unknown. If the mobilized mass is composed essentially of lymphocytes circulating in the blood, there is little hope of significantly reducing the sessile pool. On the other hand lymphocytes seen in the blood following the injection of a lymphocytosis inducing agent may be part of a newly established recirculating pool. In this case repeated injections followed by ECIB may effectively promote lymphocyte depletion and immunosuppression.

ECIB and ECIL continue to be important con-

cepts particularly in the field of transplantation. In experimental animals ECIB alone has been shown to substantially extend the survival of renal allografts in the absence of any apparent toxic effects. More importantly, studies with skin allografts suggest than ECIB may act synergistically with reduced doses of immunosuppressive drugs. In animals so treated no significant reduction in antibody synthesis to tetanus toxoid was observed.[19] In clinical renal transplantation ECIB may, therefore, allow a reduction in the dose of immunosuppressive drugs which themselves can cause widespread destruction effects on normal tissues. Recent reports in a limited number of patients indicate this is indeed true.[28] Further experimentation with ECIB and ECIL is clearly indicated.

REFERENCES

1. Heymans, J. F. Iso, hyper et hypothermisation des mammifères par calorification et frigorification du sang de la circulation carotidjuglaire anastomosée. Arch. Int. Pharmacodyn. 25:1, 1921.
2. Cronkite, E. P., Jansen, C. R., Mather, G. C., Nielsen, N., Usenik, E. A., Adamik, E. R., and Sipe, C. R. Studies on lymphocytes. I. Lymphopenia produced by prolonged extracorporeal irradiation of the circulation of the blood. Blood 20:203, 1962.
3. Cronkite, E. P., Jansen, C. R., Rai, K. R., Cottier, H., and Fliedner, T. M. The combined application of lymph duct drainage and extracorporeal irradiation of the blood in the study of lymphopoeises. *Cell Proliferation,* A Guinness Symposium, Oxford, England. Blackwell Scientific Publications, p. 126, 1963.
4. Slatkin, D. N., Jansen, C. R., Cronkite, E. P., and Robertson, J. S. Extracorporeal Irradiation of blood: Calculations of the radiation dose. Rad. Res. 19:409, 1963.
5. Cronkite, E. P., Chanana, A. D., Stoner, R. D., Schnappauf, H., Cottier, H., Jensen, C. R., and Rai, K. R. Influence of extracorporeal irradiation of blood upon lymphocytes and immunity. Colloque du C.N.R.S. sur la Greffe des Cellules Hematopoietiques Allogeniques, Paris, France, September 1964.
6. Cronkite, E. P., Jansen, C. R., Cottier, H., Rai, K. R., and Sipe, C. R. Lymphocyte production measured by extracorporeal irradiation, cannulation and labeling technique. Ann. N. Y. Acad. Sci. 113:566, 1964.
7. Kuhl, O. A., Ripperger, W., Huszagh, D. W., and Cronkite, E. P. Cobalt-60 medical fluid irradiator. Design, dose, irradiation volume and flow rate considerations and usefulness. Nucleonics 22:79, 1964.
8. Cottier, H., Cronkite, E. P., Jansen, C. R., Rai, K. R., and Singer, S. Studies on lymphocytes. III. Effects of extracorporeal irradiation of the circulating blood upon the lymphoreticular organs of the calf. Blood 24:241, 1964.
9. Cronkite, E. P., Chanana, A. D., and Schnappauf, H. Extracorporeal irradiation of blood and lymph in animals: Its effects on homografts and on lymphoma. New Eng. J. Med. 272:456, 1965.
10. Sipe, C. R., Chanana, A. D., Joel, D. D., Cronkite, E. P., and Schnappauf, H. The influence of varying dose and repetitive short sessions of extracorporeal irradiation of the blood on the production of lymphopenia. Rad. Res. 25:684, 1965.
11. Chanana, A. D., and Cronkite, E. P. Semi-permanent extracorporeal arterio-venous and thoracic duct-venous shunts in bovines. Amer. J. Vet. Res. 27:683, 1966.
12. Joel, D. D., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Schiffer, L. M., Schnappauf, H., and Stryckmans, P. A. Effects of extracorporeal irradiation of the blood on leukemit: Malignant lymphoma of cattle. Amer. J. Vet. Res. 28: 387, 1969.
13. Schnappauf, H., Joel, D. D., Chanana, A. D., DiGiacomo, R., and Cronkite, E. P. The influence of *invitro* radiation of blood on red cell survival after auto- and homo-transfusion of cells labeled with Fe^{59} or Cr^{51}. Rad. Res. 25:611, 1965.
14. Gowans, J. L. The recirculation of lymphocytes from blood to lymph in the rat. J. Physiol. (Lond.) 146:54, 1959.
15. Sipe, C. R., Chanana, A. D., Cronkite, E. P., Joel, D. D., and Schiffer, L. M. Studies on lymphopoiesis. VII. Size distribution of bovine thoracic duct lymphocytes. Proc. Soc. Exptl. Biol. Med. 123:158, 1966.
16. Cronkite, E. P., Chanana, A. D., Joel, D. D., Rai, K. R., and Schiffer, L. M. Influence of extracorporeal irradiation of the blood and lymph on lymphopoiesis and immuniryt. *Effects of Radiation of Cellular Proliferation and Differentiation,* p. 307. International Atomic Energy Agency, Vienna. 1968.
17. Ruchi, C., Cottier, H., Cronkite, E. P., Jansen, C.R., and Rai, K. R. Studies on lymphocytes XVII. Differential lymphocyte depletion in lymphoreticular organs of the calf during continuous extracorporeal x-irradiation of the circulating blood. Cell and Tissue Kinetics 3:301, 1970.
18. Chanana, A. D., Cronkite, E. P., Joel, D. D., Williams, R. M., and Waksman, B. H. Migration of thymic lymphocytes: Immunofluorescence and ^{3}HTdR labeling studies. In *Morphological and Funcation Aspects of Immunity,* K. Lindahl-Kiessling, G. Alm, and M. G. Hanna, Eds., Plenum Press, N.Y. p. 141, 1971.
19. Stoner, R. D. Cottier, H., Sipe, C. R., Chanana, A. D., Joel, D. D., and Cronkite, E. P. The effects of extracorporeal irradiation of circulating blood and thoracic duct lymph on tetanus antitoxin responses in calves. Rad. Res. 37:539, 1969.

20. Moon, H. W., Stoner, R. D., Joel, D. D., Chanana, A. D., and Cronkite, E. P. Response of lymphocyte-depleted calves to fluid tetanus toxoid. Am. J. Vet. Res. 29:1211, 1968.
21. McGregor, D. D., and Gowans, J. L. The antibody response of rats depleted of lymphocytes by chronic drainage from the thoracic duct. J. Exptl. Med. 117:303, 1963.
22. Chanana, A. D., Brecher, G., Cronkite, E. P., Joel, D. D., and Schnappauf, H. The influence of extracorporeal irradiation of the blood and lymph upon skin homograft rejection. Rad. Res. 27:330, 1966.
23. Chanana, A. D., Chonkite, E. P., Joel, D. D., and Schiffer, L. M. Skin allograft survival in calves. Influence of thymectomy and extracorporeal irradiation of blood. Arch. Surg. 98:803, 1969.
24. Joel, D. D., Chanana, A. D., Cronkite, E. P., and Schiffer, L. M. Modification of skin allograft immunity by extracorporeal irradiation of lymph. Transplantation 5:1192, 1967.
25. Chanana, A. D., Cronkite, E. P., Joel, D. D., and Stevens, J. B. Prolonged renal allograft survival: Extracorporeal irradiation of blood. Transplantation Proc. 3:838, 1971.
26. Cottier, H., Schindler, R., Bürki, H., Sordat, B., Joel, D. D. and Hess, M. W. Kinetic aspects of lymphocyte recirculation. Intern. Arch. Allergy Appl. Immunol. 41:4, 1971.
27. Jansen, C. R., Cronkite, E. P., Mather, G. C., Nielsen, N., Rai, K. R., Adamink, E. R., and Sipe, C. R. Studies on lymphocytes. II. The production of lymphocytosis by intravenous heparin in calves. Blood 20:443, 1962.
28. Weeke, E., and Sorensen, S. F. Extracorporeal irradiation of the blood: Lymphocyte transformation tests and clinical results after renal transplantation. Transplantation Proc. 3:387, 1971.

DISCUSSION

Frank P. Hungate, Battelle Pacific Northwest Labs: Do you have evidence concerning the persistence or dose-related effect on those cells which are initially nondividing? In other words, do you have any evidence that the small dose given twenty times has more or less effect than a large second dose given later?

Dr. Joel: We don't have this type of information with respect to lymphoid cells, unfortunately, because these are difficult to keep in the blood stream and we don't know how many transits they make. We do have good data with respect to cells which do stay in the blood, namely red cells. I can't answer your question with respect to cells that would divide afterwards. We do see chromosome aberrations. We haven't followed them for long periods of time, but we do see them in lymphoid cell populations. I'm sure that, in any given population of cells which makes a transit through the radiator, there are bound to be some cells which will survive, irrespective of the dose, even if you went to extremely high doses. But what the chances are of these surviving long enough to not have left the blood, to make another transit, we don't know.

PRELIMINARY DATA ON CHROMOSOME ABERRATIONS IN SWINE AND HUMAN LEUKOCYTES WITH A BRIEF REVIEW OF FACTORS AFFECTING THEIR OBSERVED FREQUENCY

F. P. Hungate and B. J. McClanahan*

Preliminary results from a study of relative sensitivities of porcine and human leukocyte chromosomes to X-rays suggest that aberration frequencies are slightly greater in human cells. Data are also reported for ^{90}Sr–^{90}Y irradiation of swine leukocytes, but comparable results of tests with human cells are not yet available.

Radiation dose and dose rate, quality of radiation as measured by linear energy transfer, and the modifying factors, oxygen and virus, are briefly discussed for their general role in modifying the frequency of aberrations observed following radiation exposure.

INTRODUCTION**

This paper discusses the effects on the cell's information system which are important in altering survival of somatic cells, i.e., effects on the chromosomes. To introduce this topic we will describe some preliminary results from a chromosome breakage study in progress in our laboratory.

In an ongoing study of effects on miniature swine of daily ingestion of ^{90}Sr, a study to be described in more detail later in this session by Dr. Ragan, we have consistently failed to observe chromosome aberrations in frequencies appreciably above those observed in unirradiated controls, even with cummulative doses calculated to be in excess of 10–20 K rads. This raised the question of the sensitivity of swine chromosomes which this study is attempting to answer in terms of the relative sensitivity of swine and human chromosomes.

METHODS

To compare the relative radiosensitivities of swine and human chromosomes, peripheral blood samples were obtained from unexposed control animals and from a female human volunteer. Leukocyte cultures were exposed to X-rays or to radiations from ^{90}Sr–^{90}Y.

Samples of heparinized whole blood, obtained under sterile conditions from female Pitman Moore swine and a female human volunteer, were allowed to sediment in the collection syringe at room temperature for approximately one hour. One milliliter of the plasma was placed in sterile plastic culture bottles and irradiated. Following irradiation, 1 ml of fetal calf serum and 5 ml of NCTC109 (Difco) containing 300 units/ml penicillin-G, 0.3 mg/ml streptomycin and phytohemagglutinin-M (Difco) were added. The cell culture was then incubated at 37° C for 45 hours at which time 0.01 mg colchicine was added to arrest mitosis in the metaphase stage, and four hours later the cells were prepared for observation essentially as described by Moorhead et al.[1] The slides were quickly blaze dried and stained with 2% Giemsa in phosphate buffer, pH 6.8. Suitable metaphases were photographed under oil immersion. A 250 kVp X-ray unit operated at 15 ma and with 1 mm copper + 1 mm aluminum filtration was used as the radiation source. Air doses were determined using Victoreen dosimeters and confirmed with LiF thermoluminescent dosimeters. The 1 ml plasma volume irradiated was contained in a 30 ml plastic culture flask. The doses used were 50, 100 and 200 R delivered at a constant rate of 11 R/min.

Beta irradiations were accomplished by mixing four millicuries of carrier free ^{90}Sr–^{90}Y (6.7 mCi/ml 0.01 N HC1) with 100 ml of the culture medium previously described and adding appropriate amounts of this solution to cell suspensions containing 1 ml of test plasma and

* Battelle, Pacific Northwest Laboratories, Richland, Washington 99352.

** This work was performed under the United States Atomic Energy Commission Contract AT(45-1)-1830.

cells, 1 ml of fetal calf serum and varying amounts of NCTC 109 such that all cultures contained 5 ml of growth medium in a total volume of 7 ml. The final cell cultures contained 7.4, 15, and 29 μCi ^{90}Sr–^{90}Y/ml. The incubation time, colchicine treatment, cell harvest and slide preparation were the same as described for the X-irradiation.

RESULTS AND DISCUSSION

Table I summarizes the data now available from the studies of *in vitro* irradiation. As expected, the incidence of chromatid aberrations in both porcine and human cells was not significantly increased by X-irradiation. This is consistent with the general observation that circulating leukocytes from normal healthy individuals are in the interphase, G_1, phase of the cell cycle. Thus aberrations scored as chromatid are presumed to have occurred spontaneously, not as a result of radiation exposure prior to stimulation by PHA. To minimize subjective influences in interpreting unstained gaps and terminal chromatid deletions these two categories were combined. Pooling the chromatid aberrations for the various exposures yielded spontaneous aberration rates of 0.018 aberrations/cell for swine and 0.044 aberrations/cell for humans. The value for human chromosomes is somewhat higher than the value of 0.014 aberrations/cell obtained by Bender and Gooch.[2] In a study on swine chromosomes, McFee et al.[3] observed a much higher chromatid aberration incidence (5% and higher) but failed to comment on possible causes of such a high value.

To avoid uncertainties of interpretation, chromosome terminal deletions were combined with achromatic lesions, and the frequency of these is linearly related to dose. The average one-hit aberration coefficients calculated from the data are 0.4×10^{-3} cell^{-1}R^{-1} for pig and 0.7×10^{-3} cell^{-1}R^{-1} for human cells. Comparable values of 1.1×10^{-3} cell^{-1}R^{-1} are reported for swine[3] and 2.4×10^{-3} cell^{-1}R^{-1} for humans.[2]

Multiple break aberrations are detected by the presence of ring configurations or multicentric chromosomes in the metaphase stage. Absence of this type aberration in both swine and human controls is consistent with their reported low frequency[1] and the small numbers of cells observed so far in this study. The two-hit aberration coefficient for human cells was 0.46×10^{-5} cell^{-1}R^{-2}. The apparent linearity of the swine data prevent calculation of a single value since proportionality with the square of the dose is anticipated. Our data give values ranging from 0.5×10^{-5} cell^{-1}R^{-2} to 0.09×10^{-5} cell^{-1}R^{-2}. Comparable values of 0.52×10^{-5} cell^{-1}R^{-2} for human[2] and 0.092×10^{-5} cell^{-1}R^{-2} for swine[3] have been obtained by others.

While there are notable inconsistencies within the present data which may be resolved as more data are accumulated, it appears that, at comparable radiation doses, aberrations occur with a lower frequency in swine than in human cells.

At this time no comparison of swine with human chromosome breakage by ^{90}Sr–^{90}Y beta radiation is possible since exposures of human leukocytes has not progressed to the point of providing results. At the highest dose of ^{90}Sr–^{90}Y, 29 μCi/ml, extensive cell death occurred, yielding an insufficient number of metaphases for study. It is interesting to note that chromatid aberrations increase with radiation dose because, in this case, the radiation is continuous with S and G_2 as well as G_1 stages irradiated. The relatively high frequency of chromatid, relative to chromosome, aberrations is similar to the *in vivo* results of Brooks and McClellan[4] following injection of large doses of ^{90}Sr–^{90}Y into Chinese hamsters.

As will be discussed later, dose rate is highly

TABLE I.—*Chromosomal Aberrations Occurring after In Vitro Irradiation*

	No. of Cells Scored		CTD* + CTG		CD* + AL		Rings + Dicentrics	
	P**	H**	P	H	P	H	P	H
X-Ray								
Control	160	94	3	4	0	0	0	0
50R	168	139	2	5	4	6	2	2
100 R	171	186	2	10	5	10	4	8
200 R	165	171	5	7	17	26	6	25
^{90}Sr-^{90}Y								
Control	133		3		1		0	
7.4 μCi/ml	152		6		5		2	
15 μCi/ml	169		40		11		1(1)***	

* CTD, chromatid deletions, CTG, chromatid gaps. CD, chromosome deletions, AL, achromatic lesions on both arms.

** P, porcine. H, human.

*** The number in parenthesis refers to a quadricentric chromosome.

significant to the production of the multibreak aberrations most apt to be detected. From the present finding that swine chromosomes are reasonably sensitive to aberration induction, it seems most probable that failure to observe aberrations in the animals chronically exposed to ^{90}Sr–^{90}Y is more likely due to the low dose rate than to insensitivity of the chromosomes.

We have not as yet verified the appropriateness of 49 hours as the time of maximum accumulation of first division metaphases in swine and humans. In fact, the recent observation of Bender and Brewen,[5] indicating the presence of two or more dividing populations in phytohemagglutinin stimulated human leukocytes, makes this evaluation of division times even more important for comparative studies such as those we have just reported.

REVIEW OF FACTORS AFFECTING ABERRATION FREQUENCY

Chromosomes Critical to Cell Survival

To comply with the symposium assignment, the following discussion will briefly identify some of the factors which modify the amount of chromosomal damage expected in studies such as those described above. Several reviews are available which cover topics relating to chromosome aberration more extensively than is appropriate here.[6–9]

Before proceeding, it may be useful to direct attention to early findings of Zirkle and Bloom [10] and Rogers and von Borstel [11] that chromosomes are the structures critical to radiation induced cell death. This evidence has been repeatedly supplemented by a wide variety of cell survival data such as that of Puck et al.[12,13] and Skargard et al.[14] showing strong correlation of survival with aberration frequencies. While it is true that radiation induced lethality in cells such as the nondividing oocyte [15] of young mammals does not appear to be explainable by chromosome breakage, most cell death following radiation correlates well with the frequency of chromosome aberration. From a variety of observations, it is evident that induction of breakage and the resulting new chromosomal associations are not of themselves lethal but become lethal when cells containing such aberrations undergo cell division. During the division process, information transfer to daughter cells is imprecise when aberrations lead to loss of acentric pieces, dicentric chromosomes with internuclear bridges, duplications, etc. As the magntiude of such irregularity increases the probability of losing or compromising information critical to survival of subsequent cell generations also increases.

Aberration Types

Chromosome aberrations have been produced by ionizing radiation both to provide material for genetic studies and to determine the manner by which aberrations are produced. If breakage occurs while the chromosomes are single, deletion type aberrations may be present in both chromatids and potentially present in both daughter cells. If the breakage occurs subsequent to the S phase, when the chromosomes are split and called chromatids, the deletion is observed in only one of the two chromatids and would be expected to affect only one of the daughter cells.

Chromosomal aberrations resulting from two breaks may involve a single chromosome in which case inversions or rings and deletions are produced. With techniques now available, inversions are not readily identified in mammalian cells since only the order, not the amount, of genetic material in each chromosome changes. Ring shapes are readily identified and are categorized as a double break type. Also easily identified as a double break type are the chromosomes having two centromeres. These result from breaks in two different chromosomes with rejoining in such a way that one of the portions has both centromeres, the other none. Equally likely are rejoinings with one centromere on each of the newly associated pieces. Recombinations involving more than one chromosome are termed translocations and, as with inversions, those combinations which result in all chromosomal materials attached to one and only one centromere have little effect on cell survival and are not readily identified. Rings and dicentrics in the metaphase stage are easily identified and signify multiple breakage.

If the events causing the breakage are independent, the frequency of these two break aberrations typically approaches proportionality to the square of the dose.

Dose and Dose Rate

Neary et al.[16] report that breakage can be induced by as little as 60 eV, while earlier work indicated a somewhat higher energy requirement.[17] Sixty electron volts is roughly equivalent to the energy involved in producing two ion pairs and this amount of energy, or slightly more, is found in the ion clusters along the tracks of γ, x, or energetic β rays. Thus one typically observes a linear response of terminal deletions (caused by a single break) to increasing absorbed dose.[18] In the data reported, frequencies of both swine and human chromosome deletions were approximately proportional to the X-ray dose. There was no effect of X-ray dose on chromatid deletions since the leukocytes were in the G_1 (interdivisional) stage of the cell cycle at the time of irradiation. The expected proportionality of rings and dicentrics, i.e., two break aberrations, with the square of the dose was observed with our human data but the response was linear with the swine chromosomes. Presumably this is merely the result of the small sample involved and we presume that with more data the frequencies of swine rings and dicentrics will relate more closely to the dosage squared.

Not all breaks result in a change in the chromosomes. Following breakage the two broken ends are in close proximity and a vast majority of breaks rejoin[19] with no residual damage or prejudice to cell survival. The importance of the proximity of the broken ends is well established[16,20] as is also the factor of time involved in restitution.[21] Since breaks remain open for limited times, it is to be expected that at lower dose rates or with fractionated doses a break may restitute before a second break occurs within the volume critical for exchanges, i.e., within 0.4–0.6 μm of each other.[16,21] This is a factor of considerable importance with metabolized isotopes such as ^{90}Sr–^{90}Y which may deliver large cumulative doses but at relatively low dose rates.

Quality of Radiation, LET

The rate at which energy is imparted to the biological system linearly along the track of a penetrating radiation is referred to as linear energy transfer (LET) and is expressed in terms of kilovolts/μm.[22] Generally X, gamma, and hard beta radiations are considered to be low LET radiations while protons, alphas, accelerated nuclei, etc., are considered high LET radiations. While two break aberrations typically are proportional to the square of the dose with low LET radiations, they are directly proportional to dose when high LET radiations are involved. In the region of the high LET track the energy released is so great that multiple chromosome breakage is highly probable for chromosomes lying within the zone in which recombinations can occur, i.e., a volume having a diameter of 0.4–0.6 μm. Since the simultaneous production of two breaks with four broken ends in close juxtaposition encourages the production of two-hit aberrations and tends to minimize restitution this accounts for some of the higher cell killing efficiency of the high LET radiations.

Oxygen

As with most biological damage, oxygen increases the frequency of chromosome breakage induced by low LET radiations. Typical data are reported by Deschner and Gray[23] who evaluated chromosome abnormalities in ascites cells exposed both *in vivo* and *in vitro*. Apparently the presence of oxygen during the exposure period encourages production of free radicals in the suspending solution, radicals themselves capable of producing chromosome breaks. Aberrations induced by high LET radiations are not influenced or are less influenced by the presence or absence of oxygen. Seemingly there is ample energy available along each high LET particle track to break any chromosomes within the rejoinable distance and additional free radicals are of no consequence. In any event, high LET radiations typically show neither an oxygen effect nor a dose rate effect.

The role of oxygen in affecting aberration frequency is not a simple one since, in addition to enhancing the frequency of break induction

by low LET radiations, oxygen, through the normally functioning oxidative metabolic systems of the cell, enhances the fusion of broken ends.[24,19] Until recently it was assumed that rejoining concerned the structural back bone of DNA. However, Wolff and Scott[25] find that inhibitors of protein synthesis keep breaks open while known DNA repair mechanisms have little affect on the fusion of broken ends, emphasizing the importance of the protein moiety of the nucleoprotein complex which makes up the chromosomes.

The observation that high LET radiations are unaffected by oxygen poses an attractive possibility to radiologists treating solid tumors whose central zones remain relatively anoxic despite all efforts to raise their oxygen tension. Thus, in typical X or gamma therapy, the cells in the center of solid tumors are anoxic and thus less damaged than are the more highly oxygenated cells on the surface of the tumor and the surrounding normal tissues. High LET radiations capable of releasing their energy in the tumor region would overcome this problem.

Other Factors

Other features such as mitotic delay,[26] selection against aberrations,[27] etc., might well be discussed but instead we will briefly refer to the observation of a persistent factor in plasma capable of increasing aberration rates in cells from normal individuals. Goh and Sumner[28] observed such a factor in plasma of individuals accidentally irradiated several years previously and Littlefield et al.[29] obtained similar results using plasma of patients receiving therapeutic irradiations. Such a factor was sought when aberration frequencies remained high over much longer post irradiation times than expected. It is difficult to imagine an effective free radical persisting over such long times. One possibility might be that viruses were involved since they are known to induce at least simple breakage.[30] Inclusion of viruses as factors important to genetic considerations is logical since knowledge of transduction clearly implicates them as part of the cell's potential information system. The extent to which they may modify aberration data has, as yet, not been evaluated but we are not aware of any evidence that multibreak aberrations such as rings and dicentrics are increased by the presence of viruses. Neither have we seen data which would force rejection of the possibility that viruses were the persistent agents in plasma inducing aberrations frequencies above those observed in controls. The interaction of radiations, viruses, and host information are subjects of intense interest and speculation is well warranted if it leads to informative experimentation.

SUMMARY

Preliminary data from studies of chromosome breakage in PHA induced leukocyte cultures suggest that the frequency of aberration in miniature swine is only slightly lower than in human cells following comparable X-ray exposures. Comparisons following ^{90}Sr–^{90}Y beta radiation are incomplete. On the basis of the X-ray study, it appears probable that failure to observe two-break aberrations, i.e. rings and dicentrics, in miniature swine chronically exposed to ^{90}Sr–^{90}Y is more probably due to low dose rate than to insensitivity of the chromosomes to radiation.

A brief review of the nature of radiation damage to chromosomes includes types of aberrations induced, effects on aberration yield due to dose, dose rate, radiation quality, oxygen, and other factors. Viruses are suggested as a possible factor in plasma causing chromosome breakage in individuals exposed years earlier to ionizing radiation.

REFERENCES

1. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A. Chromosome preparations of leukocytes cultured from human peripheral blood. Exptl. Cell Res. 20:613, 1960.
2. Bender, M. A., and Gooch, P. C. Types and rates of X-ray-induced chromosome aberrations in human blood irradiated *in vitro*. Proc. Nat. Acad. Sci. U.S. 48:522, 1962.
3. McFee, A. F., Banner, M. W., and Sherrill, M. N. Influence of animal age on radiation induced chromosome aberrations in swine leukocytes. Rad. Res. 41:425, 1970.
4. Brooks, A. L., and McClellan, R. O. Cytogenetic effects of strontium-90 on the bone marrow of the Chinese hamster. Nature 219:761, 1968.

5. Bender, M. A., and Brewen, J. G. Factors Influencing chromosome aberration yields in the human peripheral leukocyte system. Mutat. Res. 8:383, 1969.
6. Evans, H. J. *International Review Cytology.* T. H. Bourne and J. F. Danielli, Academic Press, New York, 1961.
7. Giles, N. H. *Radiation Biology,* Ed. A. Hollaender McGraw-Hill, New York, 1954.
8. Wolff, S. Some postirradiation phenomena that affect the induction of chromosome aberrations. J. Comp. Cell Physiol. 58:151, Suppl. I. 1961.
9. Brewen, J. G. Cell-cycle and radiosensitivity of the chromosomes of human leukocytes. Int. J. Rad. Biol. 9:391, 1965.
10. Zirkle, R. E. and Bloom, W. Irradiation of parts of individual cells. Science 117:487, 1953.
11. Rogers, R. W., and von Borstel, R. C. Alpha-particle bombardment of the *Habrobracon* Egg. I. Sensitivity of the nucleus. Rad. Res. 7:484, 1957.
12. Puck, T. T., and Marcus, P. I. Action of X-rays on mammalian cells. J. Exptl. Med. 103:653, 1956.
13. Puck, T. T., Morkovin, D., Marcus, P. I., and Cieciura, S. J. Action of X-rays on mammalian cells. II. Survival curves of cells from normal human tissues. J. Exptl. Med. 106:485. 1957.
14. Skarsgard, L. D., Kihlman, B. A., Parker, L., Piyara, C. M., and Richardson, S. Survival chromosome abnormalities, and recovery in heavy ion and X irradiated cells. Rad. Res. Supplement 7:208, 1967.
15. Parsons, D. F. An electron microscope study of radiation damage in the mouse oocyte. J. Cell. Biol. 14:31, 1962.
16. Neary, G. J., Preston, R. J., and Savage, J. R. Chromosome aberrations and the theory of RBE. III. Evidence from experiments with soft X-rays, and a consideration of the effects of hard X-rays. Int. J. Rad. Biol. 12:317, 1967.
17. Giles, N. H., Jr. Radiation induced chromosome aberrations in *Tradescantia* in radiobiology. Ed. A. Hollaender, McGraw Hill, 713, 1954.
18. Chu, E. H. Y., Giles, N. H., and Passano, K. Types and frequencies of human chromosome aberrations induced by X-rays. Proc. Nat. Acad. Sci., U.S. 47:830, 1961.
19. Wolff, S. Some post irradiation phenomena that affect the induction of chromosome aberrations. J. Comp. Cell Physiol. 50:151, Suppl. I. 1961.
20. Sax, K. Effect of centrifuging upon production of chromosomal aberrations by X-rays. Proc. Nat. Acad. Sci., U.S. 29:18, 1943.
21. Sax., K. The time factor in X-ray production of chromosome aberrations. Proc. Nat. Acad. Sci., U.S. 25: 225, 1939.
22. Zirkel, R. E. The radiobiological importance of linear energy transfer in radiation biology. Ed. A. Hollaender, McGraw Hill, p. 713, 1954.
23. Deschner, E. E., and Gray, L. H. Influence of oxygen tension on X-ray induced chromosomal damage in Ehrlich Ascites tumor cells irradiated *in vitro* and *in vivo.* Rad. Res. 11:115, 1959.
24. Wolff, S., and Luippold, H. E. The Biochemical Aspects of Chromosome Rejoining in Progress in Radiobiology. Proc. 4th Int. Conf. Radiobiol., held in Cambridge 1955. Eds. Mitchell, Holmes and Smith, Oliver and Boyd, 1956.
25. Wolff, S., and Scott, D. Repair of radiation induced damage to chromosomes. Exp. Cell Res. 55:9, 1969.
26. Yu, C. K., and Sinclair, W. K. Mitotic delay and chromosomal aberrations induced by X-rays in synchronized Chinese hamster cells *in vitro.* J. Nat. Cancer Inst. 39:619, 1967.
27. Sasaki, M. S., and Norman, A. Selection against chromosome aberrations in human lymphocytes. Nature 214:5023, 1967.
28. Goh, K., and Sumner, H. Breaks in normal human chromosomes: Are they induced by a transferable substance in the plasma of persons exposed to total body irradiation? Rad. Res. 35:171, 1968.
29. Littlefield, L. G., Hollowell, J. G., and Pool, W. H. Chromosomal abarrations induced by plasma from irradiated patients: On indirect effect of X-radiation. Radiol. 93:879, 1969.
30. Stich, H. F. Radiomimetric effect of oncogenic and nonocogenic human and simian adenoviruses. Symp, of Rad. Ind. Cancer, Athens, p. 21, 1969.

DISCUSSION

Marvin Goldman, University of California, Davis: (Inaudible).

Dr. Hungate: The techniques were as identical as we could possibly make them. In other words, in both cases, forty-five hours was used and I'm sure that what you're probably alluding to is that one has no basic assurance that the time of selection is appropriate to maximize the observation of the event. We have not taken time to look at the spectrum of the cell cycle.

BIOLOGICAL EFFECTS OF RADIATION FROM SIMULATED ^{238}Pu POWER SOURCES IN DOGS

F. A. Kallfelz, C. L. Comar, P. H. Craig and A. P. Casarett*

Biological effects of radiation from simulated ^{238}Pu power sources are being studied using the Labrador Retriever as the experimental animal. The simulated sources consist of a combination of ^{252}Cf (to provide the neutron component of a 30 watt ^{238}Pu source) and ^{90}Sr–^{90}Y Bremsstrahlung (to provide the additional photon flux needed). Sources are placed in the center of a spherical stainless steel capsule (333 ml volume) and the capsule is implanted in the left paralumbar fossa between the internal and external abdominal oblique muscles. Sources have been implanted which deliver 1, 5, 15, or 70 times the radiation dose expected from a 30 watt ^{238}Pu power source, and dose rates to various organs from these sources have been measured and calculated. A total of forty-eight dogs have been implanted to date. The maximum total accumulated dose in a single dog has been 37,000 Rad at 1 cm from the surface of the implantation capsule.

Clinical evaluation of implanted dogs has revealed no alterations in any of the parameters measured except for a decreased total circulating lymphocyte count and a cessation of sperm production in the 70X animals.

Six of the forty-eight animals have been killed (2-70X, 2-15X, and 2-blanks). Post-mortem examination has revealed a complete cessation of spermatogenesis and a striking reduction in lymphocyte population of the spleen and mesenteric lymph nodes in the 70X animals. Similar, though less severe lesions have been seen in 15X dogs. In addition, one 70X dog developed a mast cell sarcoma at the implant site and the 15X and 70X dogs killed after one year showed a reduction in numbers of megakaryocytes and erythropoietic elements of the bone marrow in the vicinity of the implant.

These preliminary results indicate that mammalian organisms may be able to tolerate the radiation dose incident with the use of ^{238}Pu power sources for major cardiac assist devices.

INTRODUCTION**

Over the past few years, considerable attention has been focused on the feasibility of using a radioisotope fueled power source for circulatory support systems. A number of radionuclides have been considered as possible fuels; however, it is now felt that ^{238}Pu is probably the isotope of choice for such applications.[1] Current concepts regarding the use of nuclear energy sources to power such systems include estimates that approximately a 30–50 watt plutonium-238 source might be necessary to provide sufficient power over about a ten-year period of useful life. One of the major questions concerning the eventual use of such devices in the human population regards the possible radiation hazard from such sources. We are currently investigating the biological effects of radiation from nuclear power sources which might be used to power an artificial heart.

MATERIALS AND METHODS

Animal Model

Large dogs were selected as the animal to be used in this study. In an attempt to reduce variability, purebred Labrador Retriever dogs are being used; these animals weigh approximately 30–40 kg when mature and are hence roughly one-third to one-half the size of a standard man. Since mature human beings would be the most likely candidates for implantation of such devices all animals have been allowed to mature to at least one year of age before being introduced into the study.

A spherical stainless steel capsule, 333 ml in volume, has been used as the basic implantation device. The sphere is hollow and contains a small central cavity, accessible by a screw plug, for the placement of radiation sources. A stainless steel cap is cemented over the threaded plug after placement of sources in the central cavity. The unit is then covered with medical grade silicone rubber (Silastic Medical Adhesive Type A) and sterilized under ethylene oxide before implantation.

* Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca, New York 14850.

** Supported by AEC Contract AT(11–1)3167.

Since it is the consensus that the most likely site for implantation of a power source in the human being would be the abdominal cavity, an abdominal site has been chosen for our studies. A pocket between the internal and external *oblique* muscle in the left paralumbar fossa is used for placement of the implantation capsule. A radiograph of a loaded implantation device in place in the left flank of a Labrador Retriever is shown (Figure 1).

Radiation Source

Because of the undesirable heat associated with the use of a ^{238}Pu source a mock source has been devised which provides radiation characteristics similar to those of plutonium, but has no accompanying heat. The radiations of biological concern from ^{238}Pu are photons and neutrons.[2] The composite source consists of ^{252}Cf to provide the neutron component and ^{90}Sr, ^{90}Y Bremsstrahlung sources to spike the photon flux of the ^{252}Cf up to that of ^{238}Pu.



FIGURE 1.—Radiograph of stainless steel implantation capsule, with contained ^{252}Cf and ^{90}Sr sources, in place in the left paralumbar fossa of a Labrador Retriever dog.

In order to obtain some information as to the radiation levels needed to produce effects, and yet not to make the number of animals included in the project excessively large, sources that produce the same radiation flux as would be expected from a 30 watt ^{238}Pu source (nominal, or 1X sources) as well as sources which produce five times the radiation level that would be expected from such a source (5X sources) have been used. In addition, some animals have been implanted with either 15X or 70X sources.

The dose rates from the various source configurations used in this study have been measured and/or calculated using data from studies in water phantoms and Labrador Retriever cadavers and compared with data from dosimetry studies of ^{238}Pu sources.[3] A comparison of the dose rates from our source configurations with that of a 30 watt plutonium source is shown (Table I). It can be seen that the dose from a nominal composite source is almost identical to that of the equivalent plutonium source. Dose rates to various organs and surfaces from a 70X source are also given (Table II).

Experimental Protocol

Animals have been implanted in four groups of twelve dogs each. Each group consists of four large source dogs, four small source dogs,

TABLE I.—*Dose Rate in Tissue (H_2O) from Simulated Sources Implanted in Labrador Retriever Dogs Compared to Estimated Dose Rate from 30 Watt Plutonium-238 Source (Electro-Refined Metal) under Similar Conditions*

Source Contents	Total Dose Rate (mrads/hr) at Given Distance from Surface of Capsule (cm)			Factor above Nominal
	1	5	10	
0.373 μg Cf 500 mCi Sr	1991	602	225	72
0.0745 μg Cf 100 mCi Sr	398	120	45	14.4
0.373 μg Cf 30 mCi Sr	158.4	46.5	16.7	5.7
0.0745 μg Cf 6 mCi Sr	27.6	8.1	2.9	1
^{238}Pu 30 Watt	28.2	8.6	3.2	

TABLE II.—*Approximate Dose Rates to Various Sites and Surfaces from a 500 mCi ^{90}Sr Bremsstrahlung Source and a 0.373 μg ^{252}Cf Source in an Implantation Capsule*

Site	Distance to surface of capsule (cm)	mrads/hr
Internal:		
descending colon	3	1123
spleen	6	470
liver	19	60
(L) iliac plexus	5	592
(R) iliac plexus	15	225
cecum	16	194
(R) kidney	17	194
Surface:		
(L) flank, over capsule	0.5	1532
(R) flank	16	112
lower dorsum	15	88
mid abdomen	7	225
anterior testis (front)	15	90
posterior testis (rear)	18	42
(L) popliteal space	15	28
(R) popliteal space	21	36
(L) eye	67	1.7
(R) eye	68	2.1
thyroid	51	27
(L) costal	24	35
(R) costal	27	18
sternum	27	22
upper dorsum	27	12

and four blanks consisting of dogs implanted with capsules containing no radiation sources. A summary of the four groups implanted to date is shown (Table III). At least one more group of dogs has yet to be implanted.

A number of physiological parameters have been routinely monitored on all dogs used in this study in order to maximize the probability of detecting any changes in function which might occur as the result of the radiation involved. Every dog used in the project has been submitted to a battery of clinical tests repeatedly before implantation so that a statistical evaluation of his normal range can be made. The same tests are then run periodically on each dog after implantation. Any alterations can be compared to the normal values obtained in the particular animal before implantation as well as to the population normal. A list of the routinely monitored parameters with the colony normals for each is shown in Table IV.

When killed, all animals, experimentals and controls, are subjected to an extensive postmortem examination. The examination consists of three phases: (a) gross inspection of organs and tissues; (b) histological examination of all lesions observed during gross necropsy (in addition to specifically radiosensitive organs and tissues regardless of gross appearance); and (c) radiographic examination of the entire skeleton.

TABLE III.—*Total Accumulated Dose From Date of Implant to February 1, 1972, at 1 cm from Surface of Capsule in Labrador Retriever Dogs Implanted with Simulated ^{238}Pu Power Sources*

Group #	Sources (X nominal)	No. of Dogs	Implant Year	Accumulated Dose at 1 cm from Surface (Rads)	Kill Year
I	70X	4	1969	37,300	?
	15X	4	1969	7,450	?
	Blanks	4	1969	—	?
II	5X	4	1970	1,820	1972
	1X	4	1970	350	1972
	Blanks	4	1970	—	1972
III	5X	4	1970	1,365	1975
	1X	4	1970	260	1975
	Blanks	4	1971	—	1975
IV	5X	4	1971	680	1974
	1X	4	1971	130	1974
	Blanks	4	1971	—	1974

RESULTS

A total of forty-eight dogs have been implanted to date (Table III). Group I dogs were implanted in October and November 1969, Group II in September and October 1970, Group III in December 1970 and January 1971, and Group IV in June and July 1971. The antici-

TABLE IV.—*Physiological Parameters Monitored in Labrador Retriever Colony*

Parameter	Normal Value	Units	S.D.
Blood studies			
Total WBC	12863	per mm^3	1791
Packed cell volume	43	%	3.3
Differential WBC			
Bands	0.2	%	0.3
Segmenters	62	%	5.6
Lymphocytes	25	%	3.7
Monocytes	7	%	1.1
Eosinophils	6	%	4.0
Serum Electrolytes			
Sodium	173.7	meq/L	10.5
Potassium	7.1	meq/L	0.8
Calcium	11.0	mg %	0.6
Serum Proteins			
Total protein	6.4	gms %	0.6
A/G ratio	1.50	—	0.26
Serum Enzymes			
SGOT	42	S-F U/ml	13.5
SGPT	46	S-F U/ml	18.6
Serum Lipase	0.4	S-T U/ml	0.2
Prothrombin Time	9.8	seconds	0.8
Blood Sugar	82	mg %	9.0
Blood Urea Nitrogen	18	mg %	2.0
Serum Creatinine	0.94	mg %	0.17
Sperm Count	302,000.	per mm^3	197,000.

pated kill dates for these animals are also shown (Table III). However, actual kill dates may be changed depending upon clinical results observed.

Results of clinical testing of animals in the study have indicated changes in only two of the parameters under observation. Both of these changes have occurred only in the large source dogs of Group I, i.e., 70X animals. Beginning about 2–3 months after implantation, total lymphocyte counts were observed to decrease from the preimplantation normal of about 2,000 per cubic millimeter of blood. The decrease continued over the next year until the lymphocyte count had fallen to approximately 50% of the control value, where it has remained since that time. Secondly, examination of semen collected beyond three months after implantation from the male 70X animals revealed a virtual absence of spermatozoa in the ejaculate. No consistent change from the normal has been observed in any of the other animals at this time.

Of the forty-eight animals implanted to date only six have been killed. The six animals killed comprise half of Group I animals, i.e., two 70X animals (1 after three months and 1 at one year after implantation), two 15X animals (1 after four months and 1 at one year after implantation), and two blanks (both at one year after implantation).

Post mortem examination of these animals was performed as outlined. One of the most striking lesions was seen in the lymphoid tissue. There were severe reductions in the lymphocyte populations of the white pulp of the spleen in the 70X animals. Similarly there were reduced populations of small and medium lymphocytes in the mesenteric lymph nodes of the two 70X animals and in the 15X dog killed after one year. In these animals there was an almost total ablation of the germinal centers of the lymph nodes.

All four of the irradiated male dogs had gonadal lesions. These consisted of altered cell populations within seminiferous tubules with morphologic evidence of reduction and qualitative change in spermatogenic sequences. In three dogs there appeared to be graded sub-normal levels of spermatogenesis while in one animal (a 70X dog killed after 1 year) there was a complete cessation of spermatogenesis. In addition, an intratubular seminoma was found in the testes of one 15X dog. Since this lesion occurs quite frequently in the dog its presence cannot be interpreted as a radiation effect.

In the two blank and two 15X animals, the implantation capsule was surrounded by a thin capsule of dense fibrous connective tissue 0.5—1 mm thick with an incomplete inner lining of mesothelioid cells. On the outer surface of the fibrous capsules was a loose meshwork of areolar tissue which suspended the device between the muscle layers. Tissues immediately adjacent to the capsule seemed quite normal in all respects. The connective tissue layer surrounding the implantation capsule in the 70X dog killed after three months was intensely reactive; this was interpreted as due to chronic infection since a fistula had developed in this dog shortly before it was terminated.

The implantation site of the 70X dog killed after one year was also intensely reactive. The inferior aspect of the connective tissue capsule was distorted by a large spherical nodule (approximately 5 cm in diameter) which proved histologically to be a mast cell sarcoma. The nodule was intimately adherent to the capsule, but the overlying skin was not attached. In addition to the nodule, much of the tissue capsule was infiltrated with nests of anaplastic mast cells, and a metastasis was present in the ipsilateral lumbar lymph node.

Technically satisfactory marrow specimens were available from the four dogs killed after one year. The 15X and 70X dogs showed an altered cell population characterized by a reduction in megakaryocytes and erythropoietic elements and an apparent predominance of large, relatively immature, and undifferentiated cells. These changes occurred in the marrow of bones immediately adjacent to the implantation capsule.

DISCUSSION

Assuming a mid-abdominal dose of roughly 300 mrads/hr (extrapolated from Table II), the mesenteric lymph nodes and spleen probably received a dose of about 630 rads in three months which was sufficient to cause the de-

creased population of lymphocytes and atrophy of lymphoid germinal centers seen in the 70X dog killed at this time. Such changes were also seen after one year or 2,400 rad but were not significantly greater in severity at this time than at three months. Similar, though less severe lesions were seen in the 15X animals.

The testicular lesion amounted to almost complete cessation of spermatogenesis in the 70X animal killed at three months after implantation and aspermatogenesis in the 70X dog killed after one year. The total dose to the testes during three months was 140 rads while after one year the total accumulated dose was 570 rads assuming a dose rate of 65 mrads per hour. Such changes would probably be expected from such a dose at a daily dose rate of approximately 1.5 rads.[4]

Of great interest is the mast cell tumor found in association with the connective tissue capsule in the 70X dog killed at one year. This region (within 1 cm of the implant surface) received a total dose in the neighborhood of 18,000 rad during the year of implantation. It is true that mast cell tumors occur frequently in dogs, and it might be argued that the occurrence of the tumor in this animal is fortuitous. However, the tumor did show some unusual characteristics, specifically: 1) mast cell tumors are rare in the Labrador Retriever;[5] 2) spontaneous mast cell tumors arise almost without exception in the dermis, or deep layers of the skin, while the tumor in question had no association with the skin; 3) the tumor metastasized and this occurs only rarely in cases of spontaneous mast cell tumor; and 4) the tumor arose at the site of maximum radiation dose. Because of these special features it is felt that this tumor may be an example of radiation carcinogenesis; however, no definite conclusions can be made on the basis of this one case.

The decreased level of erythropoietic elements and megakaryocytes in the marrow of the lumbar vertebrae immediately adjacent to the capsule was associated with a dose of approximately 170 rads in the 15X dog and 750 rads in the 70X dog killed after one year.

Only one other group has conducted studies similar to the one in progress at our laboratory.[6–8] Their results to date are very similar to the results reported here. However, they have detected fewer pathological changes than seen in the current study. This is probably due to the fact that their sources are in the probable range of 1X to 5X, and pathological data are given for animals killed after two years. Based on our results, two years may be too short a time period for the accumulation of sufficient dose for distinct biological effects from sources in this dose rate range.

SUMMARY

Results obtained to date from this study, though preliminary, are encouraging. It should be emphasized that all clinical and pathological changes seen have been in 15X or 70X animals. No functional changes have been discerned in 1X or 5X animals. It is true that the 15X and 70X animals have been implanted for a longer period of time than any of the other dogs and have accumulated a much larger total dose. However, clinical changes were seen in the high dose rate animals at a much earlier time (3 months) than in the lower dose animals some of which have now been implanted for one and one-half years. Because of the relatively low dose rate in the 1X and 5X animals, long term studies, i.e., five to ten years, will probably be necessary in order to detect possible subtle changes such as life-shortening and shortening of incidence times for various tumors.[9]

REFERENCES

1. Cole, D. W., Mott, W. E., and Sagan, L. A. Factors relating to the applications of radioisotopes to circulatory support systems. Isotopes and Radiation Tech. 7:138–145, 1969.
2. Mullins, L. J., and Leary, J. A. Plutonium-238 for biomedical applications. Nuclear Applications 6:287–297, 1969.
3. Cross, F. T., and Sheppard, C. In-phantom dosimetry of Plutonium-238 heat sources in relation to circulatory support systems. AEC Research and Development Report, BNWL-1489, 1970.
4. Casarett, A. P. *Radiation Biology*, Prentice-Hall, Englewood Cliffs, N.J., 1968.
5. Post, John E. Personal Communication.

6. Norman, J., Pegg, C., Sandberg, G., and Lee, R. Effects of intracorporeal heat and radiation on dogs. Proc. Art. Heart Program Conf. 901–911, 1969.
7. Huffman, F., Sandberg, G., and Norman, J. Studies of the effects of additional endogenous heat. N.I.H., N.H.L.I. Report TE4064-115-71, 1971.
8. Sandberg, G., Molokhia, F., Ponn, R., Huffman, F., and Norman, J. Studies of long-term effects of implanted discrete heat and radiation sources in dogs and primates. Proc. Natl. Conf. On Res. Animals in Med., N.I.H., N.H.L.I., Washington, 1972.
9. Wallburg, H. E. Animal radiation data and their relevance to man. Proc. Natl. Conf. On Res. Animals in Med., N.I.H., N.H.L.I., Washington, 1972.

OBSERVATIONS ON CHROMOSOMAL MORPHOLOGY DURING SIMULATED INTRACORPOREAL RADIATION FROM NUCLEAR-POWERED ARTIFICIAL HEARTS

Farouk A. Molokhia, William J. Robinson, Fred N. Huffman and John C. Norman*

The study of chromosomal aberrations may offer a useful means of evaluating the effects of exposure to ionizing radiation. Chromosome studies were carried out in (1) two dogs with implanted 16 and 24-watt ^{238}Pu sources for 25 and 26 months, respectively, (2) four dogs with thoracic radiation equivalent sources (RES) comparable to 24-watt ^{328}Pu sources but with negligible heat output for periods up to three years and continuing, (3) two dogs with abdominal RES capsules for 16 months and continuing, (4) surviving offspring of RES-bearing dogs for periods up to three years and continuing, and (5) three primates with abdominal RES comparable to 50-watt ^{328}Pu capsules for six months and continuing. Peripheral lymphocytes were cultured and prepared for karyotyping by the technique of Moorhead et al.[7] Fifty mitoses were scored to detect aberrations in each preparation. Some chromosomal anomalies were noted in cultures early in the observation period. These included chromosome gaps, breaks, rings and fragmentation, and chromatid gaps. Studies later in the observation period, however, showed normal chromosomal morphology in all radioisotope-bearing animals. The offsprings of RES parents also showed normal morphology and karyotypes, although the fetuses were 5–14 cm from the RES capsules during two months of gestation. Taken together, the accumulating data from multiple cultures over a three-year period suggest that prolonged intracorporeal irradiation of the magnitudes employed does not affect chromosomal morphology as determined by the techniques employed.

INTRODUCTION**

A totally implanted nuclear-powered artificial heart or circulatory assist device would expose the patient to intracorporeal reject heat and radiation. Because the potential dangers have not yet been thoroughly documented, we have been studying the long-term effects of intracorporeal heat and radiation in experimental animals. Plutonium-238 (^{238}Pu) heat exchangers with thermal outputs of 16 and 24 watts were implanted in dogs.[1] At the same time, we have been studying effects of continuous intracorporeal radiation in primates as well as in dogs at neutron and gamma exposure dose rates equivalent to 24 and 50 watt Plutonium-238 heat exchanger sources but with negligible thermal outputs.[2,3] Radiation from the latter source is equivalent to projected clinical requirements for a proposed implantable nuclear-powered circulatory-assist device.[2,3]

The production of chromosomal aberrations by ionizing radiation offers a useful means of diagnosing radiation exposure.[4] In general, in the absence of complicating factors, an increase in chromosomal aberrations can be taken as an indication of radiation exposure.[5] Under some circumstances, the frequency of chromosomal aberrations may even be used as a "biological dosimeter" to provide quantitative estimates of the magnitude of exposure.[6]

The purpose of this paper is to report hematologic and chromosomal data obtained to date from animals subjected to intracorporeal radiation for as long as 36 months.

MATERIAL AND METHODS

Implantation of Radioactive Capsules

Blood-cooled nuclear-powered heat exchangers were implanted in series with the descending thoracic aorta in two mongrel dogs. A 16-watt ^{238}Pu capsule was implanted in one, an 85-pound female (Plutina, #3779). She was monitored for 25 months, at which point she succumbed to pulmonary embolism, apparently unrelated to prolonged intracorporeal heat or

* From the Department of Surgery, Harvard Medical School; the Cardiovascular Division, Sears Surgical Research Laboratories, Harvard Surgical Unit, Boston City Hospital and the Thermo Electron Research and Development Center, Waltham, Massachusetts.

** Supported in part by N.I.H. contracts PH 43-66-982 and PH 43-67-1116.

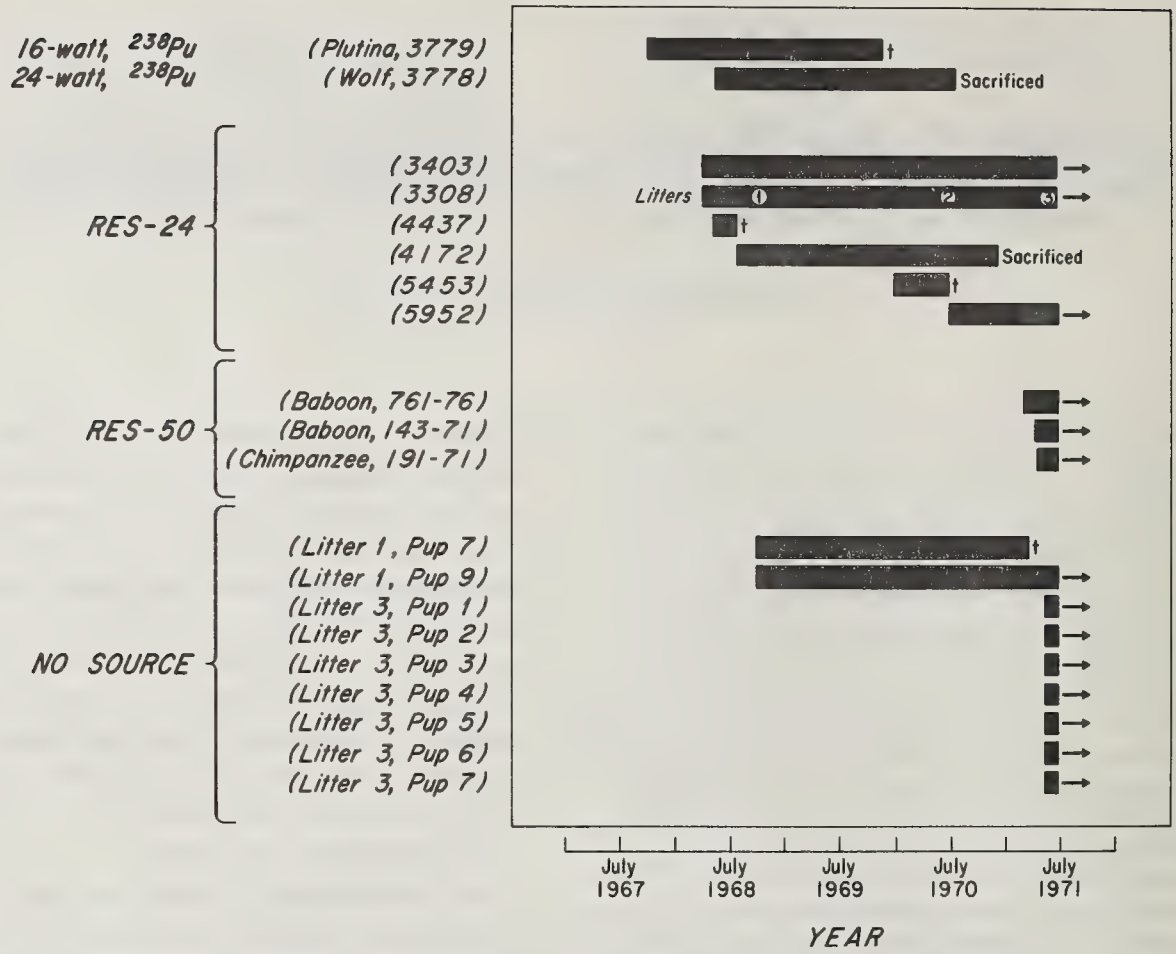


FIGURE 1.—Summary of radioisotope-bearing animals and their offspring.

radiation. A 24-watt ^{238}Pu capsule was implanted in the other dog, a 90-pound male (Wolf, #3378), who remained in good health until he was sacrificed 26 months later (Figure 1).

The blood passing through the 16-watt ^{238}Pu heat exchanger was exposed to a radiation dose of 60 mrem/hr while the 24-watt ^{238}Pu heat exchanger had a blood radiation dose of 90 mrem/hr.

Composite radiation equivalent sources (RES) of negligible heat output but of radiation dose rates comparable to 24-watt ^{238}Pu capsules were implanted in six other dogs. In four of the subjects (#3403, 3308, 4437 and 4172) the sources were placed in the left costophrenic pleural space, and in two (#5453 and 5952), they were implanted in the left iliac fossa. Two of the dogs in this group died of pneumonia (#4437 and 5453), and one was sacrificed at 14 months (#4172).

RES's of radiation dose rate comparable to 50-watt ^{238}Pu capsules were implanted in the left iliac fossa of two baboons (#761–70 and 143–71) and one chimpanzee (#191–71).

Table I shows the nuclear radiation characteristics of all the radioisotope sources used thus far in the study.

Nine surviving pups from three litters of offspring from RES dogs 3403 and 3308 were also studied (Figure 1). Radioactive capsules were not implanted in the puppies. However, by being 5 to 15 cm from the mother's radioisotope source, they had been exposed during the two-month gestation period to 5–170 mrem of neutron and gamma radiation per hour.

Monitoring

Blood tests were done before implantation of the radioactive capsules and were repeated periodically during the months that followed. RBC, WBC, differential leukocyte, platelet and reticulocyte counts, and red cell indices were determined.

TABLE I.—*Nuclear Radiation Characteristics of Radioisotope Sources*

Nuclear Source Identification	Pu-238 16 watt	RES-24 Stainless steel attenuator	Pu-238 24 watt	RES-50 Tantalum attenuator
Neutron source strength (Neutron/sec.)	1.02×10^5	1.20×10^5	1.50×10^5	2.40×10^5
Neutron dose rate at 8 cm. (MREM/hr.)	11.0	13.2*	20.3	32*
Photon dose rate at 8 cm. (MREM/hr.)	9.0	19.0	14.0	50
Total dose rate at 8 cm. (MREM/hr.)	20.0	32.2	34.3	82

* Assumes fission and AM-24/Be neutrons to be dose equivalent.

Lymphocytes were cultured for 72 hours and prepared by a slightly modified version of a method described by Moorhead et al.[7] Most scoring of aberrations was done visually with the high-power, oil-immersion objective of the microscope. The slide was scanned under low magnification ($\simeq$ 100 X), and when suitable figures were found they were scored at higher magnifications. As a rule, 50 mitotic figures were scored in each preparation. Crude karyotype analyses were done visually. When it seemed appropriate, a more complete analysis was made from an enlarged photomicrograph of the chromosome spread. Any count of chromatid breaks below 2% was considered normal on the basis of control slides. Occasionally fluorescent microscopy karyotyping was used in the primate group studies.

RESULTS

Blood values of all the animals remained within normal limits. Representative data from some of the RES dogs are given in Figure 2.

Results of the chromosomal studies are summarized in Figure 3. Despite meticulous care in carrying out Moorhead's technique, some preparations were not usable. Three months after

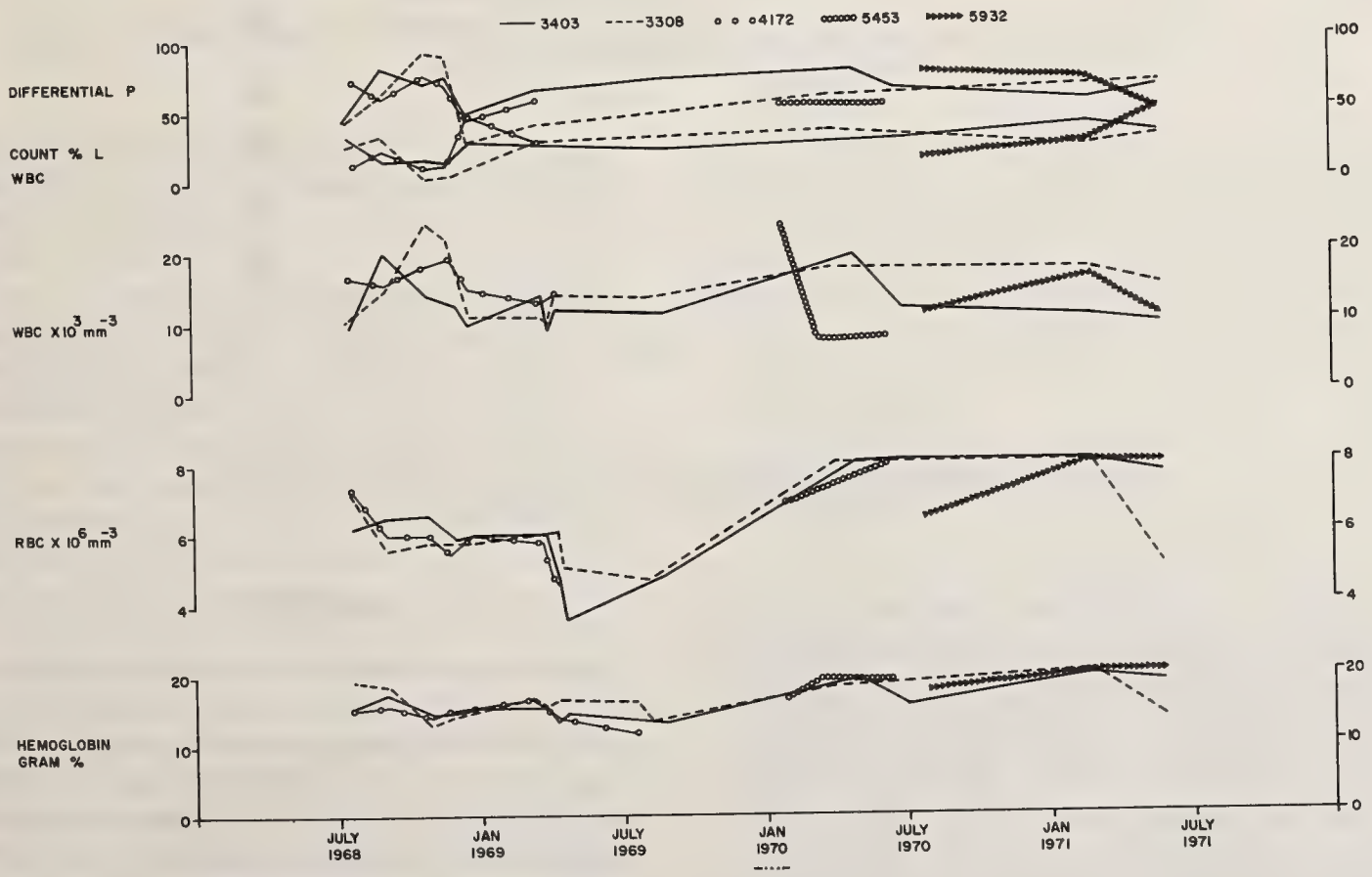


FIGURE 2.—Hematologic data from dogs implanted with radioactive sources.

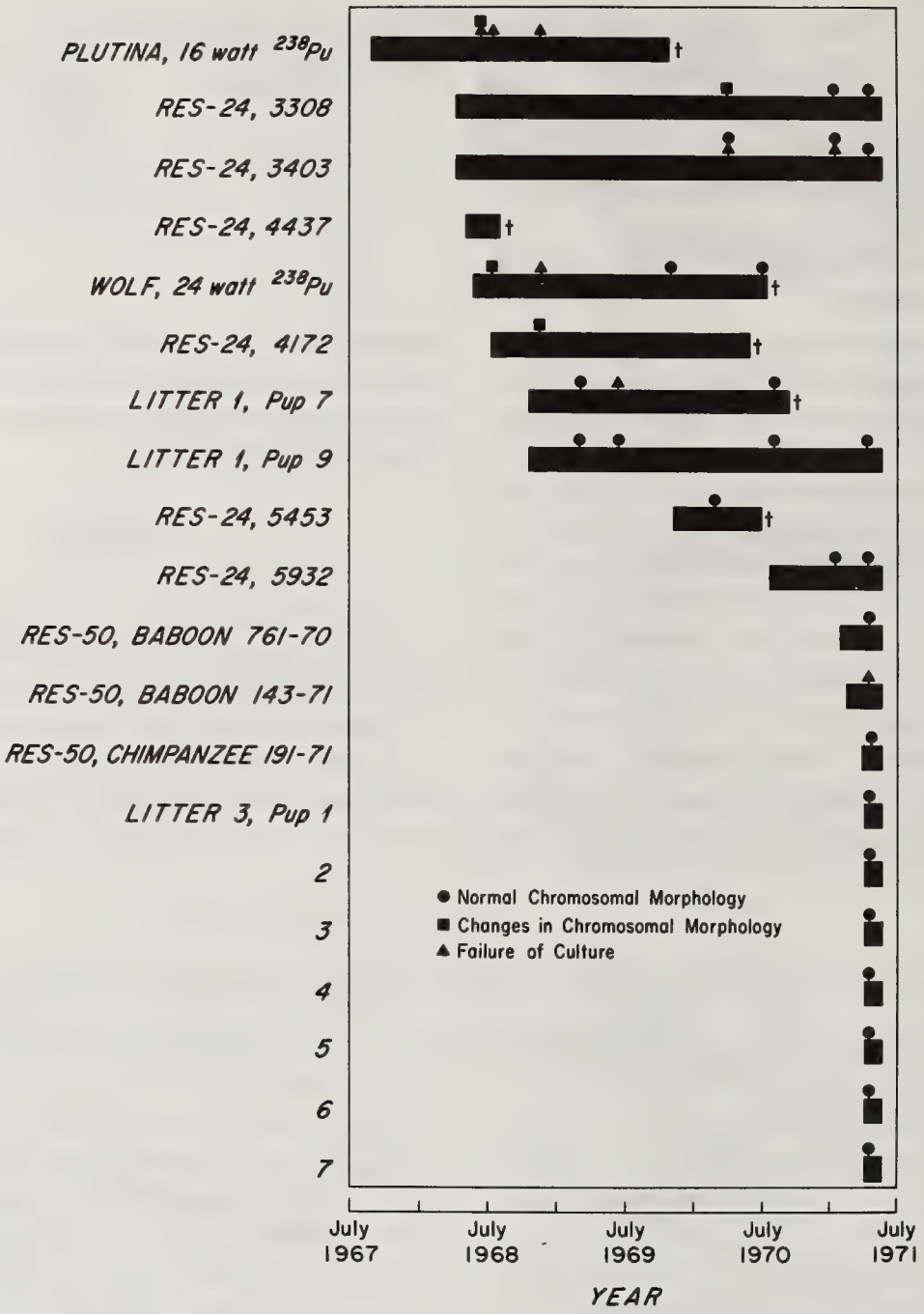


FIGURE 3.—Summary of chromosomal morphology data from radioisotope-bearing animals and their offspring.

implantation, cultures from four dogs (Plutina, Wolf, RES #3308 and 4172) showed chromatid gaps and chromosomal breaks, gaps, fragmentation and rings (Figures 4 and 5). Later cultures, however, showed normal metaphase figures and normal karyotypes of these subjects. The rest of the animals, including the offspring, showed normal chromosomal morphology and karyotyping throughout the study (Figure 6).

DISCUSSION

Kozinets[8] reviewed the literature on the effects of ionizing radiation on human lymphopoiesis and lymphocytes and concluded that the absolute number of lymphocytes in the blood is a sensitive "biological" indicator. Studies of the hematologic effects of continuous exposure to radiation at dose rates ranging from 3 to 10 rad/day have revealed that the lymphocytes



FIGURE 4.—Chromosomal pattern of a dividing lymphocyte. Arrow indicates a fragmented chromosome. (oil immersion field x 1000 Giemsa stain).

are affected more dramatically than other cells; after about 500 days of irradiation, the lymphocyte count will fall to between 20 and 50% of normal values.[9]

In our study, no significant hematologic changes occurred as a result of the prolonged intracorporeal irradiation. Because of the appreciable natural fluctuations in peripheral blood count, however, it would be difficult to demonstrate statistically significant changes in cell counts at these low dose rates. Furthermore, it is not possible to extrapolate to lower dose rates in order to estimate the possible effects of an implanted radioisotope-powered artificial human heart or pacemaker with perfected radiation shielding.

Polyploidy, endoreduplication and the frequency of aneuploid cells, in addition to chromosomal aberration frequencies, have been suggested as cytogenetic indices of radiation exposure. Unfortunately, though, only chromosomal aberration frequencies seem to be sufficiently stable, reproducible and predictable to be of practical value for radiation diagnosis.[6] In our study, the only chromosomal changes that might have been caused by the irradiation were the fragmentation and the ring formation found soon after implantation. For several reasons, however, these findings must be interpreted cautiously.

For instance, even in the cells of normal, unirradiated people, chromosomal aberrations occur from time to time. These "spontaneous" aberrations constitute the background "noise" or base line above which increases caused by radiation exposure are measured, and they include all the types of aberrations which may be induced by radiation, though in some cases at a much lower frequency. Spontaneous aberration frequencies thus set a lower limit on the radiation dose that can be detected by cytogenetic analysis.[6] Furthermore, many environmental and health factors other than radiation exposure—drugs and viral infections, for ex-

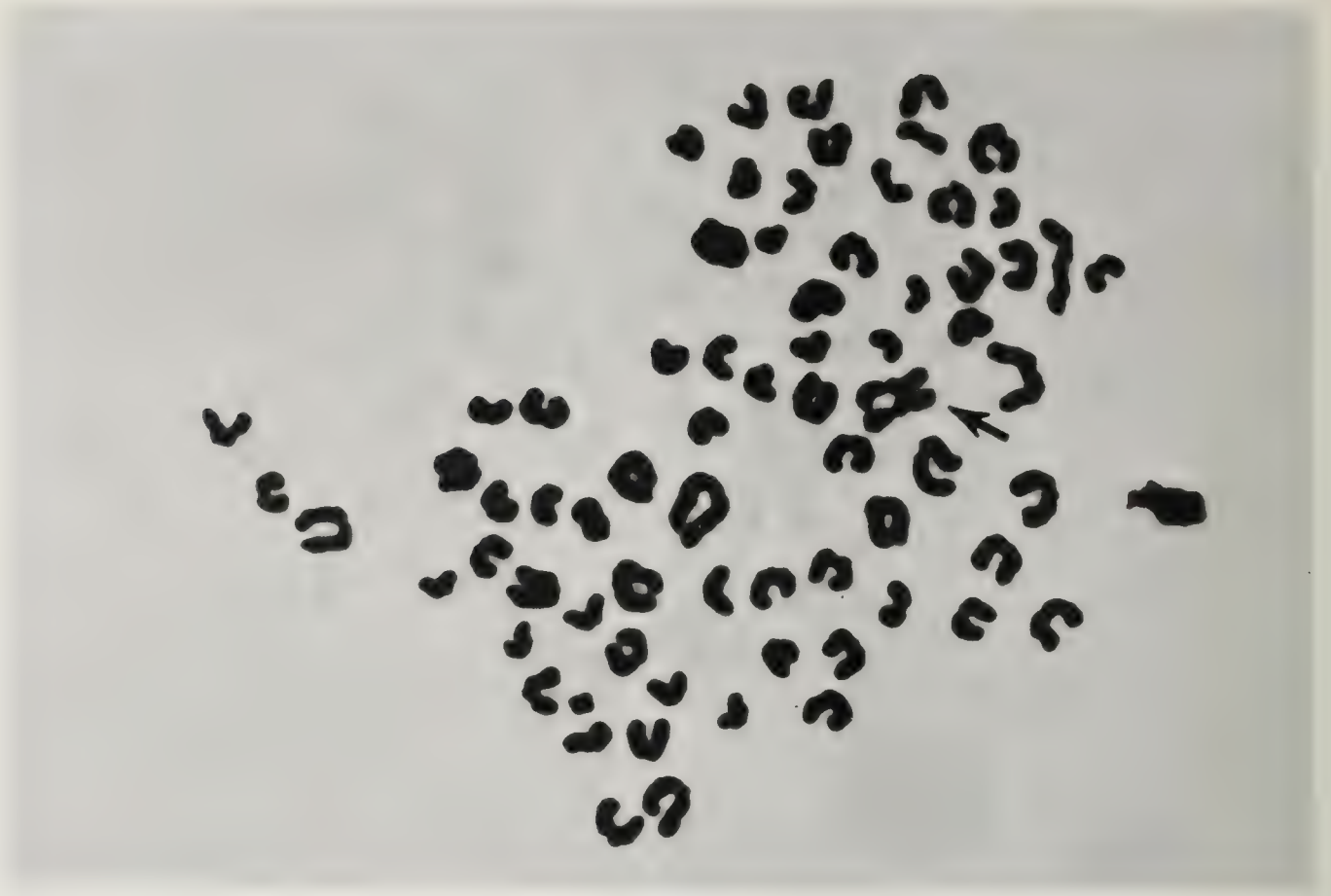


FIGURE 5.—Chromosomal pattern of a dividing lymphocyte. Arrow indicates a chromosomal break. (oil immersion field x 1000 Giemsa stain).

ample—can increase the frequency of chromosomal aberrations.[10]

The peripheral leukocytes that divide in response to phytohemagglutinin *in vitro* do not normally divide while in the circulation. They are, in fact, pre-DNA-synthesis cells in which only chromosome-type aberrations are induced by irradiation[5]—an advantage for the purpose of radiation diagnosis. The problem of aberrations arising in culture is minimal because most such aberrations will be chromatid types and easily distinguished (with the exception of the non-sister-union isochromatid deletions).

Prolonged exposure to low doses of ionizing radiation would not ordinarily produce an appreciable number of multiple-break aberrations, but when the source is implanted intracorporeally, the irradiation may actually produce more multiple-break aberrations than single-break aberrations because of the irregular distribution of the radioactive material within the body. In other words, some cells may be exposed to high dose rates, even though the average dose rate for the entire body may be very low. Thus, circulating cells may be irradiated in transit near the source and then recirculated to more distant fixed populations. It has been estimated that the true number of small lymphocytes might be forty times the number of circulating lymphocytes and that for each lymphocyte entering the blood through the thoracic duct there are 4–12 lymphocytes from other sources.[11] Other factors are lymphocyte life span and kinetics, the degree of fibrous tissue formation and cellular degeneration around the radioactive capsule, leading to relative ischemia of the area most exposed to radiation.

Tough et al. suggested that x-ray induced chromosomal aberrations in the peripheral blood may decrease within a few months.[12] Bender and Grooch found chromosomal aberrations persisting for three years in eight patients who had been accidentally over-exposed to radioactive material.[13] Those cases, however,

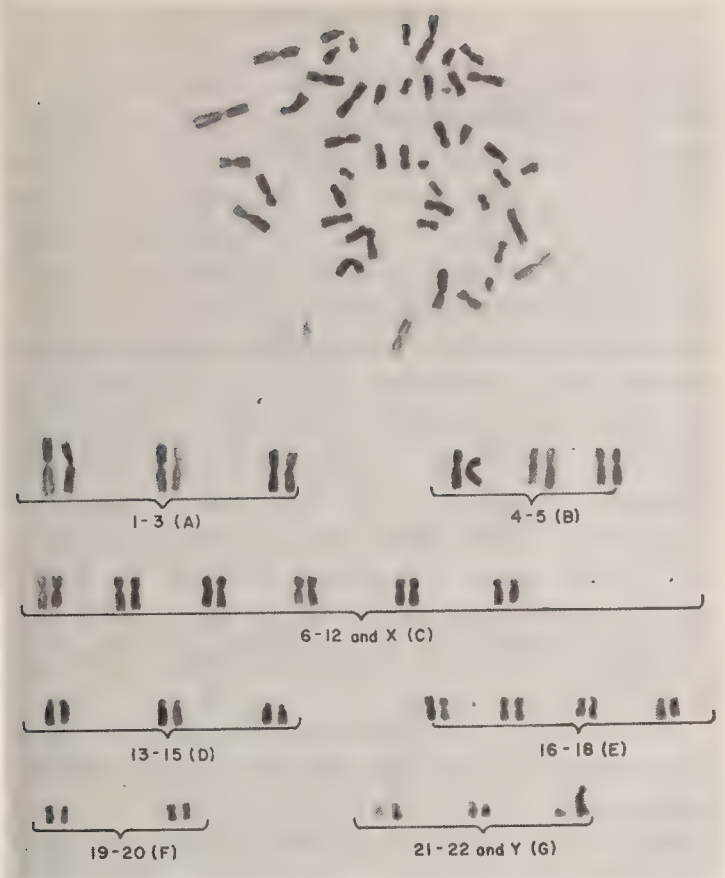


FIGURE 6.—Karyotyping of male chimpanzee #191–71. Forty eight normal chromosomes. (oil immersion field x 1000 Giemsa stain)

involved acute high dosage exposures. The decrease of abnormal cells with time is probably related to the fact that damaged cells would have difficulty completing normal mitosis and therefore would end in what Kaplan[14] termed "mitotic suicide". However, the decrease in abnormal forms may be more apparent than real since the loss of a fragment during a later cell division may make a cell appear normal unless a full karyotype is made. It is also possible that abnormal cells are selectively destroyed by the reticuloendothelial system.

Although the most important chromosomes from the point of view of long-term human radiation hazard are those of the germ cells (the meiotic chromosomes), these were not analyzed in the current study. However, if chromosomal abnormalities had affected the germ cells, they would have been passed on to the next generation. Monitoring of the offspring from irradiated parent dogs showed good mitotic activity and normal karyotyping.

It is essential to ensure that aberration frequencies are determined only during first division cells. According to Sasaki and Norman,[15] this would necessitate the use of cultures grown for about 48 hours at 37°C because by 72 hours, some cells reach their second or third *in vitro* division which alters the observed frequency of aberrations. However, since the 72-hour period of culture was used in the beginning of this long-term study, it was retained to facilitate comparisons.

In conclusion, transient chromosomal aberrations were noted in a colony of radioisotope bearing canines three months after implantation. Later studies of this group and of radioisotope-bearing primates showed normal chromosomal morphology. Studies of offspring suggested that no chromosomal abnormalities were transmitted or effected.

SUMMARY

Radioactive capsules simulating the dose rates for proposed and developing implantable artificial hearts were implanted in eight adult dogs and three adult primates. Serial hematologic profiles, lymphocyte cultures and chromosomal morphologic studies were carried out for three years to determine the effects of intracorporeal radiation. Nine Puppies, sired by a capsule-bearing father and subjected to irradiation from the mother's capsule during gestation, were also studied. No changes in blood values were detected. Chromosomal abnormalities were observed in four dogs, three months after source implantation, but later studies revealed normal chromosomal morphology in all animals and their offspring.

REFERENCES

1. NORMAN, J. C., COVELLI, V. H., BERNHARD, W. F. and SPIRA, J. Implantable nuclear fuel capsules for an artificial heart. Trans. Amer. Soc. Artif. Int. Organs. 14:204, 1968.
2. SANDBERG, G. W., JR., HUFFMAN, F. N. and NORMAN, J. C. Experimental observations of intracorporeal strontium 90-americium 241/beryllium sources simulating radiation fields from nuclear-powered artificial hearts. Ann. Thorac. Surg. 9:401, 1970.

3. Ponn, R. B., Molokhia, F. A., Huffman, F. N., Curtis, G. W. and Norman, J. C. Abdominal implants of radiation sources. Preliminary report. J. Surg. Res. 11:207, 1971.
4. Lea, D. E. *Actions of radiations on living cells.* 2nd ed., Cambridge University Press, Cambridge, p. 189, 1955.
5. Bender, M. A. and Grooch, P. C. Chromatid-type aberrations induced by x-rays in human leukocyte cultures. Cytogenetics, 2:107, 1963.
6. Bender, M. A. The use of chromosome analysis in the diagnosis of radiation injury. Chapter 25 in *Manual of radiation haematology—A joint undertaking by IAEA and WHO.* Technical Reports series No. 123. International Atomic Energy Agency, Vienna, p. 277, 1971.
7. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M. and Hungerford, D. A. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell. Res. 20:613, 1960.
8. Kozinets, G. Radiation effects on the lymphocytic cell renewal system. Chapter 11 in *Manual on radiation haematology—A joint undertaking by IAEA and WHO.* Technical Reports series No. 123. International Atomic Energy Agency, Vienna, p. 133, 1971.
9. Ingram, M. and Mason, W. B. *Biological effects of external radiation.* McGraw-Hill, New York, p. 253, 1954.
10. Eggen, R. R. *Chromosome diagnostics in clinical medicine.* Charles C. Thomas, Springfield, Illinois, p. 137, 1965.
11. Cronkite, E. P., Bond, V. P. and Conrad, R. A. *The hematology of ionizing radiation, atomic medicine.* Williams and Wilkins, Baltimore, p. 192, 1964.
12. Tough, I. S., Buckton, K. E., Barkie, A. G. and Court-Brown, W. N. X-ray induced chromosome damage in man. Lancet 2:849, 1960.
13. Bender, M. A. and Grooch, P. C. Persistent chromosome aberrations in irradiated human subjects. Rad. Res. 18:389, 1963.
14. Kaplan, H. S. Cellular effects of ionizing radiation. Bull. N. Y. Acad. Med. 36:649, 1960.
15. Sasaki, M. S. and Norman, A. Selection against chromosome aberrations in human lymphocytes. Nature 214:502, 1967.

DISCUSSION

McClellan G. Blair, ARCO Nuclear Co.: I have a question regarding the area surrounding the implants. What was the nature of the effect of the capsule on the surrounding tissue?

Dr. Molokhia: Some of the dogs which survived at 14 and 25 months were subjected to a detailed histological examination. On examination, the area of the capsule was surrounded for the first few millimeters by degenerating acellular tissue. Then, it was dense fibrous tissue. The tissue was examined by Doctor Shields Warren, Professor of Radiation Pathology in Harvard Medical School, and he could identify some large fibroblasts, which he called radiation fibroblasts. But there was no evidence of any neoplasia or tumors anywhere in the dogs at 25 months.

Dr. Blair: Were there any neoplasia or anything like this formed on the interior of the tubes used?

Dr. Molokhia: No viable cells could be seen.

Dr. Blair: What was the temperature gradient between the blood and the body of the capsule?

Dr. Molokhia: Between 43 and 37 C.

Dr. Blair: I think you reported it as an increase in the temperature of the blood cells following the implantation?

Dr. Molokhia: No, I'm speaking about the lung further off. Once the animals had recovered from the surgical intervention, the temperatures dropped to normal.

Dr. Blair: But the temperature of the surrounding tissue would have to be somewhat higher than the temperature of the blood.

Dr. Molokhia: Right. That was 41 to 42 C, as compared with the normal.

PATHOLOGIC EFFECTS OF CHRONIC ^{90}Sr INGESTION IN MINIATURE SWINE

H. A. Ragan, P. L. Hackett, B. J. McClanahan and W. J. Clarke*

Over 700 female miniature swine, extending through three generations, have been exposed to daily feedings of from 1 to 3100 μCi ^{90}Sr. In addition, there were 200 untreated, female, littermate controls. Body burdens of ^{90}Sr reached approximately 7.5 and 10 times the daily intake in the original and F_1–F_2 generations, respectively, with the soft tissue radiation doses ~ 1/1,000 that for bone.

Although radiation osteitis was a common finding, only 7 pigs had evidence of 11 individual bone tumors, with >80% occurring in the skull. These tumors were classified morphologically as osteosarcomas (73%) or giant cell tumors (27%).

The most consistent disorders associated with chronic ^{90}Sr feeding were effects on the hematopoietic system. At levels >25 μCi/day (5,000–20,000 rads accumulated skeletal doses), there occurred a progressive decrease in circulating leukocytes and platelets, and a precipitous, terminal fall in erythrocytes, with many of these animals dying of hemorrhagic diatheses. A second syndrome, observed particularly in animals at the 125 and 625 μCi/day levels, was a broad spectrum of myeloproliferation ranging from myeloid metaplasia to frank blast cell leukemia.

INTRODUCTION**

In attempting to define the potential hazards of ^{90}Sr to man, there have been many animal studies performed over the past 30 years, utilizing numerous animal models to evaluate the biologic damage. Interest in ^{90}Sr arose because of its relative abundance as a long-lived (28 year radioactive half-life) radionuclide present in nuclear fallout. ^{90}Sr, by emission of an 0.54 MeV beta particle, decays to ^{90}Y, which in turn emits a 2.27 MeV beta particle with a 64 hour radioactive half-life. Since strontium is metabolized in a manner similar to calcium, it is deposited primarily in, and excreted slowly from, the skeleton, thereby producing relatively high radiation dose rates to osseous tissue and bone marrow.

The majority of animal studies have utilized single or multiple injections of radioactive strontium in rodents or dogs. This mode of administration, as opposed to ingestion, results in uneven skeletal distribution, producing areas of high strontium concentrations and severe focal bone damage. Because of their small bone mass, rodents lose approximately 70% of the ^{90}Sr radiation energy from bone.[1] This energy loss in man and miniature swine was found to be 12%[1] and 13%,[2] respectively.

To simulate the most probable route of ^{90}Sr entry and its distribution in man, our laboratory initiated a study in 1958 using miniature swine exposed to ^{90}Sr by daily ingestion. This species was selected because it is an omnivore with a mature weight and bone mass similar to man, and an estimated life span in excess of 12 years.[3] This study, coupled with a similar experiment at the University of California at Davis using beagle dogs, was designed to supplement the rodent studies and provide the data necessary for extrapolation of the biohazards of ^{90}Sr to man.

MATERIALS AND METHODS

The experimental design for this study has been previously reported in detail.[4] Briefly, the original generation female Pitman-Moore miniature swine were started on a daily ^{90}Sr feeding regimen at 9 months of age and subsequently bred to a control boar to produce the F1 generation, which in turn produced the F2 generation. Thus, the F1 and F2 offspring were exposed to ^{90}Sr in utero, from their dam's milk, and then by daily feeding. Weaning was accomplished at 6 weeks of age, at which time the animals were started on daily ^{90}Sr feeding at 1/4

* Biology Department, Battelle, Pacific Northwest Laboratories Richland, Washington 99352.

** This work supported under Contract AT(45-1)-1830 with the U. S. Atomic Energy Commission.

their dams' level. This was increased by 25% of the destined ^{90}Sr dose every six weeks, so that by 6 months of age they were at full level. This regimen permitted the dietary calcium-strontium ratio to remain constant during the rapid growth of adolescence.

Over 700 miniature swine representing 3 generations have been exposed to ^{90}Sr at 1, 5, 25, 125, 625, and 3100 μCi/day over the past 13 years. In addition, there have been approximately 200 female controls.

A second major group, comprising 47 miniature swine, have been exposed to ^{90}Sr at 5 different periods: (1) in utero only, (2) nursing only, (3) in utero and during the nursing period only, (4) from conception throughout adult life, and (5) from the nursing period and throughout adult life (Table I). The dams, and those offspring continuing on ^{90}Sr, received 125 μCi/day. The offspring are being held for lifetime observation.

The animals were kept in groups of 3 to 6 swine per pen within controlled temperature buildings. Individual feeding stalls were utilized within each pen so that strict dietary control could be maintained. The ^{90}Sr chloride solution fed these pigs was absorbed into a large feed pellet which was fed with the morning ration.

Blood samples were obtained at intervals from the anterior vena cava using commercially available evacuated tubes.* Routine hematologic and serum biochemistry parameters were determined using the Coulter Model B, and more recently the Coulter Model S, and the Technicon Autoanalyzer. Methods of analysis have been previously reported.[5] Bone marrow samples were aspirated, (without the use of anticoagulants), from the sternum using a Rosenthal needle.

About 250 animals have been killed at various ages for the express purpose of determing the tissue distribution of the deposited ^{90}Sr, and to evaluate the early effects of the radionuclide ingestion. The remainder have been maintained for lifetime observation and killed as their clinical condition dictated. At death, complete gross and histologic examinations were performed on all tissues and autoradiograms made on selected cases.

Cytogenetic studies were performed by standard methods on peripheral leukocytes and aspirated bone marrow.[6]

* Becton-Dickinson Vacutainer

TABLE I.—*Experimental Design Uterine-Milk Exposure Study*

GROUP	DAM	Sr^{90} EXPOSURE	NO.
RUCF	125 μCi	IN UTERO ONLY	10
CURN	CONTROL	NURSING ONLY	10
RURN	125 μCi	IN UTERO & NURSING	8
CURF	CONTROL	NURSING & FEED	10
RUF_1	125 μCi	IN UTERO, NURSING & FEED	9

RESULTS

Radiation Dose

The methods of dosimetry and assays for skeletal ^{90}Sr accumulation in miniature swine ingesting 1 thru 625 μCi ^{90}Sr per day from birth to approximately 8 years of age have been reported by Palmer et al.[2] Original sows started on daily ^{90}Sr at 9 months of age accumulated a ^{90}Sr body burden approximately 7.5 times the daily intake, whereas the F1 and F2 generations reached about 10 times their daily intake. Less than 1% of the absorbed ^{90}Sr was located in soft tissue, so that the radiation dose to extraosseous tissue was approximately 1/1,000 that received by bone. The radiation received by the ovaries was essentially the same as other soft tissues.

Thermoluminescent dosimeters were used to determine the radiation dose to fetuses at various periods during gestation. At approximately midway in gestation (55 days), the dose rate to the fetuses of dams ingesting 125 μCi/day was approximately 28 mrad/day, whereas at term a mean dose rate of approximately 50 mrad/day was obtained. The osseous tissue dose rate at 55 days was approximately 50 times that received by soft tissue, and 500 times greater at term. There was no significant maternal contribution to the radiation dose received by the fetal thymic area. The accumulated radiation to a term fetus would be about 50 rads from a 125 μCi/day dam, and approximately 400 mrads from a 1 μCi/day dam.

Farrowing Performance

An extensive statistical analysis of the farrowing performance of the animals ingesting ^{90}Sr has been previously published.[7] Except for weaning weights of offspring from the original 625 μCi/day dams, there were no differences in liter size, percentage of stillborn, birth weights, or weaning weights between control and animals ingesting up through 625 μCi/day. The lower weights of 625 μCi/day offspring were probably due to the severe radiation effects produced in the dams resulting in reduced lactation during the nursing periods and to the accumulative radiation effects on the neonates.

Farrowing parameters in the F1 generation dams, ingesting 0 through 125 μCi/day, were indistinguishable, with the exception of larger litters in the 25 μCi/day group. The reason for this difference is not apparent.

Original sows of the 3,100 μCi level failed to survive the gestation period, and 625 μCi/day offspring died before sexual maturity, so that there are no F1 nor F2 generations, respectively, in these groups.

It is important to note that the lowest level fed these swine (1 μCi/day) results in a body burden approximately 5 times higher than the International Commission for Radiation Protection body burden limits for occupational exposure in man,[8] and is 2,000 times greater than the highest ^{90}Sr level ever reported in American diets.[9] Even at levels 125 times these values, there were no adverse affects noted on farrowing performance.

Life Span Effects

Currently there are 78 swine still alive that were assigned to the original study. These include thirty-two controls, twenty-eight 1 μCi offspring, eleven 5 μCi offspring, and two 25 μCi original sows and five of their offspring. The results of cumulative mortality data, though incomplete at this time, indicate increased deaths in original pigs of the 625 and 125 μCi/day groups after 1 and 5 years, respectively, and possibly after 10 years in the 25 μCi/day group.[10] Cumulative mortality in the offspring of the 1, 5 and 25 μCi/day groups was not significantly greater than control values through 9 years of age. The high mortality rates in the 125 and 625 μCi/day groups were due to the extreme stresses placed on the hematopoietic system by ^{90}Sr. The vast majority of deaths, or cases requiring euthanasia, in all groups over 5 years of age were due to uterine tumors or faulty dentition.

The mortality rates in the uterine milk exposure study are extremely interesting. The animals that have received ^{90}Sr exclusive of the gestation period, that is only during the nursing period and then by feeding, had a 70% mortality by 36 months of age, whereas those exposed to ^{90}Sr in utero, nursing, and by subsequent feeding experienced only a 22% death loss over the same time period. The one death in the group exposed in utero only, was a non-radiation related acute necrotizing pancreatitis.

Osseous Lesions

Radiographically, there were no definitive lesions at ingestion levels less than 125 μCi/day. At 125 μCi/day, radiolytic areas became apparent in the mandible after approximately 4 years of ^{90}Sr ingestion. The same was true of 625 μCi offspring taken off ^{90}Sr feeding and allowed to survive 2 to 3 years. In these higher dose levels, there occurred rather severe periodontal osteonecrosis resulting in early loss of teeth and/or suppuration. These observations were not surprising since greater than 30% of the ^{90}Sr body burden was located in the skull,[11] partially due to a greater ^{90}Sr deposition per gram of bone in this area.[12]

Histologically, the ^{90}Sr induced bone lesions consisted of irregular calcification and an increase in resorption cavities, reactive bone, and fibrosis, progressing to severe osteonecrosis at ^{90}Sr levels of 625 μCi/day and above. A detailed analysis of histologic changes by dose levels has not been made at this time due to diminished bone effects at the lower dose levels. As the 1, 5, and 25 μCi/day groups accumulate a greater radiation dose, a difference may be detectable if the survival time is sufficiently long.

Although a high incidence of bone tumors was predicted as an end result of ^{90}Sr feeding, this has not proved to be true. Only seven animals in the chronic toxicity study have devel-

oped detectable bone tumors. Five of the animals were 125 μCi/day offspring, and two of these had multicentric tumors. They had received ^{90}Sr throughout their life and died at 744–1210 days of age. The remaining two animals with bone neoplasia were 625 μCi/day offspring that received 156 μCi ^{90}Sr/day for approximately 6 weeks after weaning and then were removed from further radionuclide ingestion for 1,093 and 1,561 days before death. Accumulated skeletal radiation doses ranged from 9,000–15,000 rads in both the 125 and 625 μCi offspring. Nine of the eleven bone tumors were located in the head region, and one each in a rib and ulna.

Histologically, 73% of these tumors were classified as osteosarcomas, 27% as giant cell tumors or osteoclastomas, and in most cases they appeared to arise from the periosteal surface. A detailed description of these tumors has been previously reported.[12]

Chromosomal Effects

Bone marrow cells obtained from animals at the higher dose levels (625 and 125 μCi/day) contain a greater number of one-break abnormalities than are observed in the control swine. However, no consistent chromosomal abberations have been noted in those animals that have developed hematopoietic neoplasms.

Hematopoietic Effects

Peripheral Blood. The effects of chronic ^{90}Sr ingestion on the peripheral blood values are best demonstrated at the highest feeding level, i.e., 3100 μCi/day (Figure 1). Four weeks after 9-month-old animals were started on ^{90}Sr ingestion, the platelet, segmented neutrophil, and lymphocyte values were reduced to approximately 50% of the preexposure levels. These continued a steady decline until death occurred approximately 3 months later. There was a lag in erythrocyte response, compared with other cellular elements, and the effect was less pronounced until near the time of death. Erythrocyte depression was, however, similar in magnitude to that observed in leukocytes and platelets. These animals failed to survive the gestation period so there was no F1 generation for study.

A similar sequence of events was observed with 625, 250 and 125 μCi ^{90}Sr/day, although the magnitude of change and time of death shifted to a longer interval as the daily ^{90}Sr feeding level was reduced. Original animals started on 625 μCi/day survived 9–12 months, so were able to produce an F1 generation, which if maintained on ^{90}Sr after weaning, survived only about three months.

The F1 generation, 250 μCi ^{90}Sr/day, animals experienced pronounced depression in platelets and leukocytes, and 6 of 20 died of a hemorrhagic syndrome by 7 months of age. With the exception of two animals, ^{90}Sr feeding was discontinued at 200 days of age. This resulted in partial recovery of peripheral blood leukocytes and platelets. One of the animals removed from further ^{90}Sr feeding developed a neoplasia characterized by an abnormal serum protein and plasmacytoid cells in the blood and marrow, which has been tentatively diagnosed as multiple myeloma.

At 125 μCi/day, original animals lived 4–6 years with moderate development of thrombo-

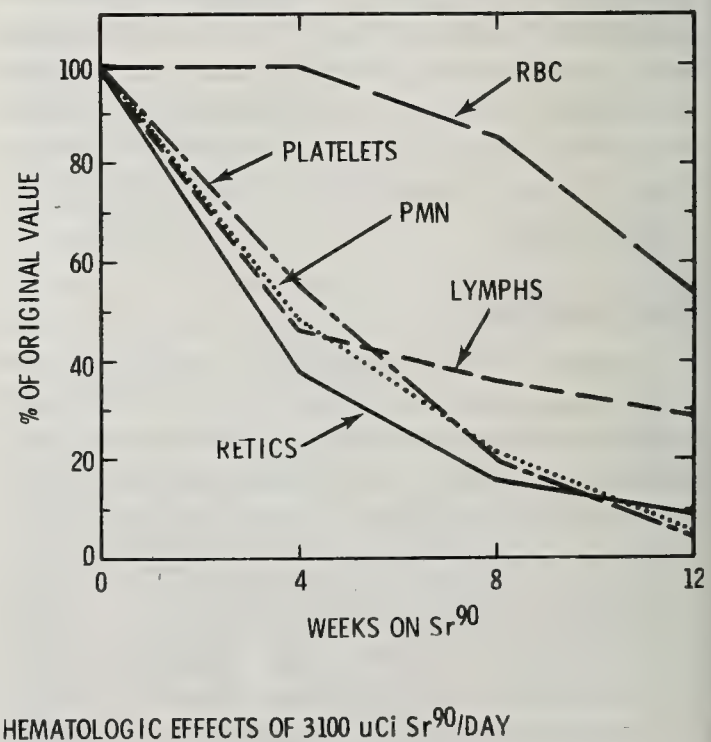


FIGURE 1.—Hematologic Response of a Miniature Pig Ingesting 3,100 μCi ^{90}Sr/day.

cytopenia, neutropenia and lymphopenia. Their offspring manifested much more severe hematopoietic depression, so that by one year of age their leukocyte and platelet values were approximately 50% that of control animals, and deaths occurred at about three years of age. Many animals in this group developed hematopoietic neoplasia which will be detailed below.

In the 1, 5 and 25 μCi/day levels, there have been no definitive changes observed to date in the circulating cellular elements of either original or offpsirng pigs. There was a suggestion of a slight depression in neutrophil values in the 25 μCi/day group, but this observation awaits confirmation by statistical analysis. As the animals in these groups age beyond 5 years, evaluation of hematologic data becomes complex because of complications from uterine tumors, oral infections, and other causes of leukemoid reactions.

In the uterine-milk exposure study, the peripheral blood values of animals kept on 125 μCi/day have been consistent with those previously observed at that level. Cellular elements of those exposed in utero only, milk only, or in utero and milk only, have not been different than those of the controls (Figure 2). Examination of aspirated sternal bone marrow when these animals were 33 months of age revealed a shift to the left in the maturation curves of both the erythroid and myeloid series in those animals that continue to ingest ^{90}Sr (Figures 3 and 4).

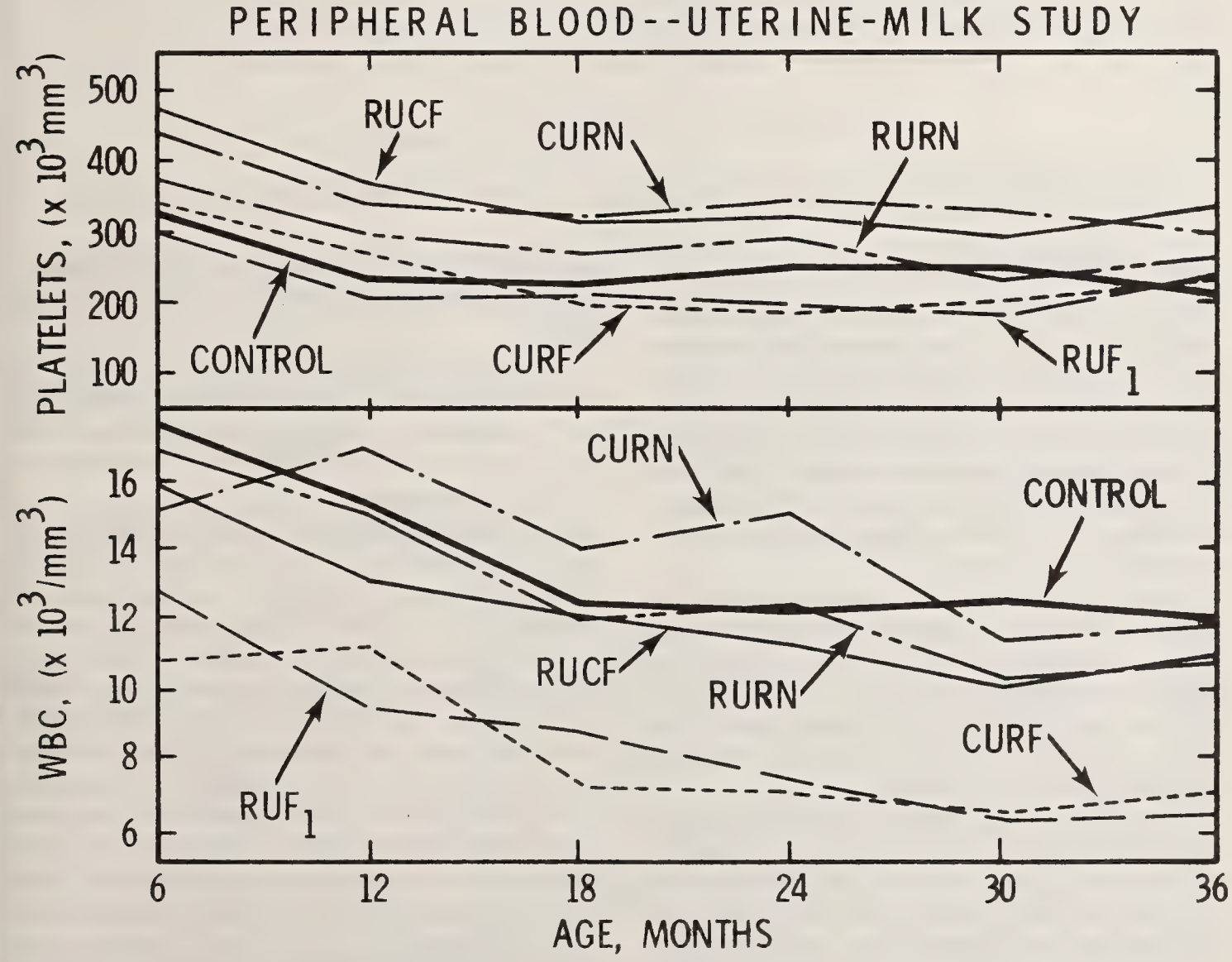


FIGURE 2.—Peripheral Leukocyte and Platelet Values of Miniature Swine in the Uterine-Milk Exposure Study.

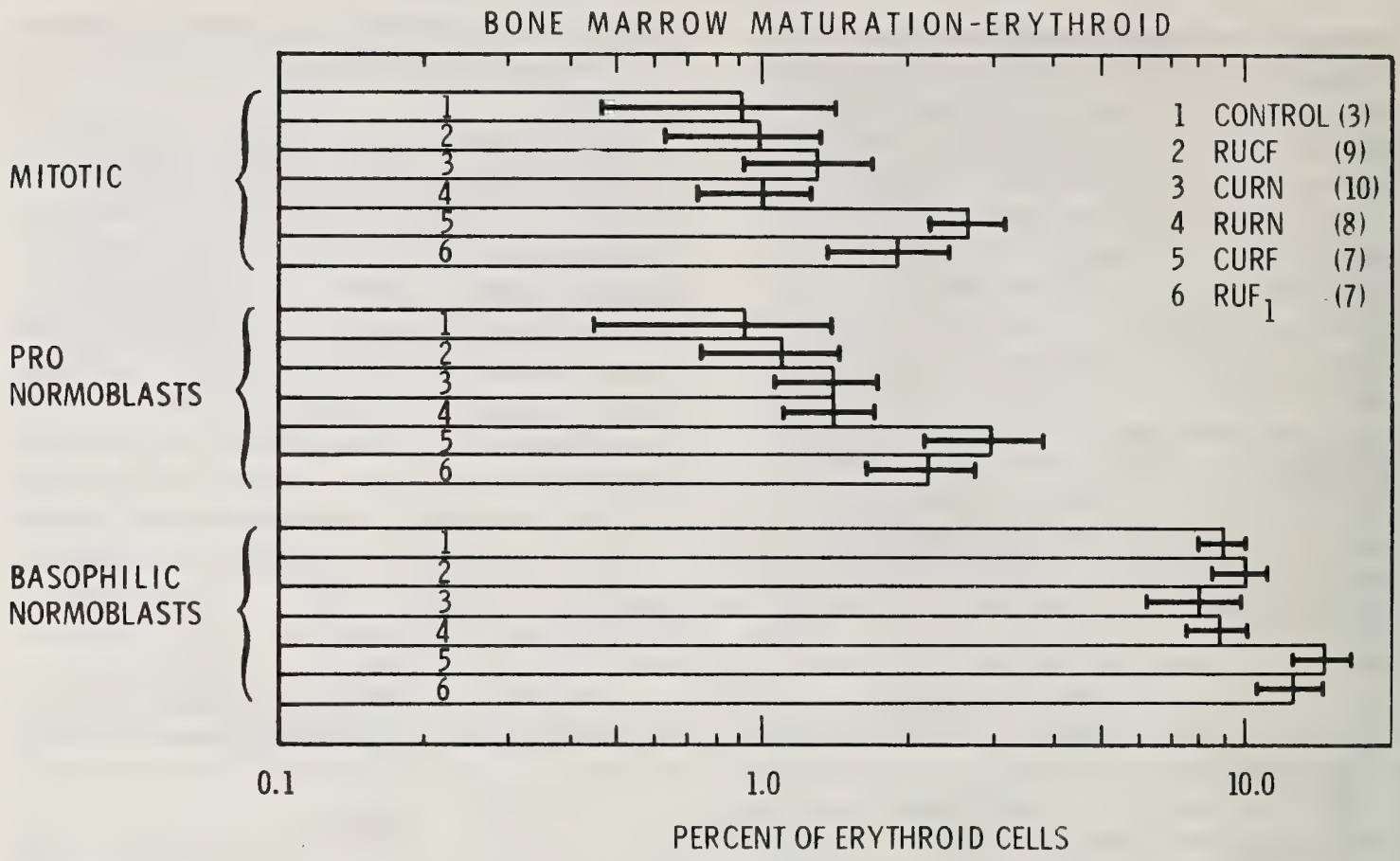


FIGURE 3.—Pertinent Erythroid Marrow Maturation in Uterine-Milk Exposure Study (Mean ± S.E.).

Hematoproliferative Disorders

The spectrum of proliferative disorders observed in animals at the various dose levels is shown in Table II. The histopathology of these dyscrasias has been previously described.[13] At the 3,100 μCi/day level two of three animals developed areas of myeloid metaplasia that were most evident in the liver, spleen, lymph nodes and kidneys, but with scattered foci in the myocardium and adrenals.

The 625 μCi/day offspring experienced an incidence of myeloid metaplasia of approximately 50%. In addition, there was one case each of myeloid and lymphoid neoplasia. Most animals in this group died of an acute hemorrhagic syndrome associated with profound thrombocytopenia, leukopenia and anemia.

At the 125 μCi/day level, only 5 of 42 animals considered at risk developed myeloid metaplasia. However, the incidence of hematopoietic neoplasia in the parents was approximately 40% and in the offspring 70%, with the mean age at death being about 6 and 3 years, respectively. Of these, 61% were considered myeloid, 28% lymphoid and 11% stem cell proliferations.

With lower accumulated radiation doses (1, 5, and 25 μCi/day), the incidence of metaplasia, and particularly neoplasia, was reduced and there was a greater tendency for production of lymphoid neoplasms.

The hematopoietic neoplasms generally developed relatively late in the particular lifespan for each dose level, and after accumulated radiation doses of 6,000 to 20,000 rads, the majority occurring at approximately 15,000 rads. Clinically, these neoplasms were characterized by being very acute in nature with only a few weeks between the presence of blast forms in the peripheral blood and death of the animal. About one-half of the myelogenous leukemia cases had leukemic peripheral blood values. The majority of the myelogenous leukemias were classified as granulocytic, with one each of eosinophilic, basophilic, DiGuglielmo's disease, and two myelomonocytic forms.

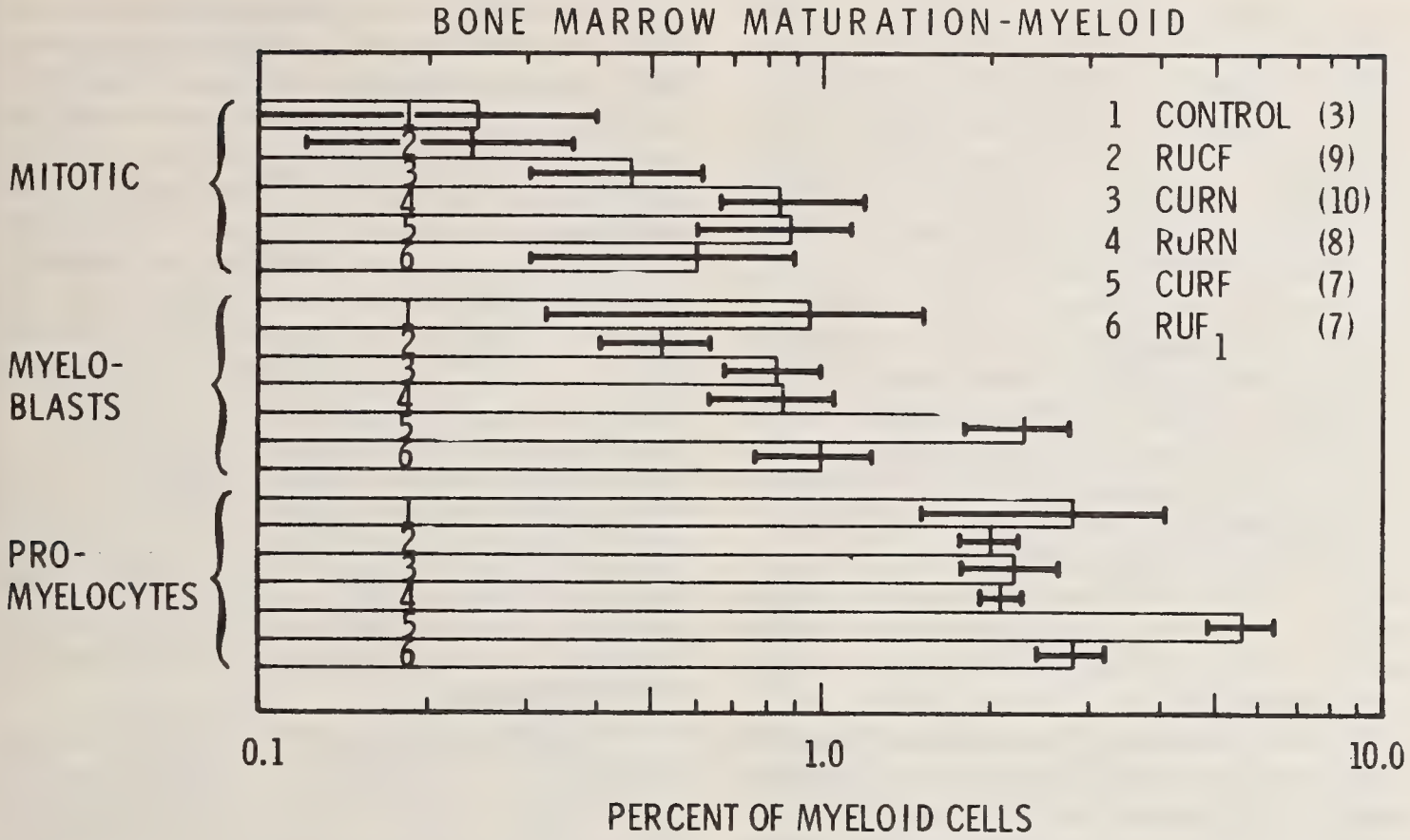


FIGURE 4.—Pertinent Myeloid Marrow Maturation in Uterine-Milk Exposure Study (Mean ± S.E.).

There were two cases of myelogenous leukemia observed in 62 control animals at risk. Curiously, both of these animals developed eosinophilic forms. There have been no cases of lymphoma observed in our control population.

DISCUSSION

Although significant postnatal radiation effects of ^{90}Sr have been manifested at several high dose levels, there was no evidence of increased fetal or neonatal mortality even at accumulated fetal radiation doses of greater than 150 rads. Since the fetus receives little radiation from maternal tissues, and ^{90}Sr is a bone-seeking radionuclide, the sensitive fetal tissue would be well differentiated prior to the time a significant ^{90}Sr concentration would begin accumulating. This does not, however, presume that bremstrahlung radiation from maternal

TABLE II.—*Hematoproliferative Dyscrasias in Swine Ingesting ^{90}Sr*

^{90}Sr Level (μCi/day)	Generation	Number of animals at risk	Age at death (months)*	Average rad dose (x 10^3) to skeleton*	Lymphoid neoplasms	Myeloid neoplasms	Stem cell neoplasms	Myeloid metaplasia
3100	Parent	3	12–13	7–10	—	—	—	2
625	Parent	6	17–19	6–9	—	—	—	2
	F_1**	28	3–34	2–10	1	1	—	15
125	Parent	10	66–88	13–17	1	3	—	3
	F_1, F_2	32	24–57	10–20	7	14	3	2
25	Parent	12	54–123	2–6	—	2	—	—
	F_1, F_2	32	50–93	3–6	2	1	—	5
5	Parent	0	—	—	—	—	—	—
	F_1, F_2	24	82	1.0	1	—	—	—
1	Parent	17	92–96	<0.04	1	—	—	1
	F_1, F_2	59	52–81	<0.2	4	—	—	1
0		62	103–105	—	—	2	—	1

* For those animals with hemoproliferative disorders.
** Removed from ^{90}Sr feeding at 3 months of age.

tissues during the early period of the embryo could not result in an embryonal rest of neoplastic propensity which might become manifest under proper stimulation later in life. Vaughan,[14] in a review of the literature, concluded that even at the relatively low dose rates of diagnostic procedures the incidence of leukemia in exposed fetuses is increased.

Comparison of radiation-induced deaths in the uterine-milk exposure study are particularly interesting. The greater than 3-fold increase in mortality in those exposed to ^{90}Sr during nursing and then to radioisotope feeding seems too much to be coincidental. It is interesting to postulate the development of a relatively ^{90}Sr resistant cell population during embryogenesis, or simply a homeostatic increase in the hematopoietic stem cell compartment which would require a greater accumulated radiation dose for depletion.

The low incidence of bone tumors in swine chronically ingesting ^{90}Sr is in direct contrast to the high bone neoplasia observed in rats and dogs injected with ^{90}Sr.[15,16] The latter method of administration results in uneven skeletal distribution and areas of high irradiation intensity or "hot spots," which result in severe necrosis and tumor development in these areas. Generally, the bone tumors that developed in our swine were also in sites of most severe osteonecrosis, where extensive destruction and active remodeling processes were occurring.

Of particular interest is the observation that in those cases where it was possible to determine the site of origin, the swine bone tumors appeared to arise from the periosteal surface. Following injection of ^{90}Sr in several other species, the bone tumors originated from the endosteal surface,[17] and in a study utilizing a single intravenous ^{90}Sr injection in miniature swine it was established that greater damage occurred to the endosteum and trabeculae than to the periosteal surface.[18] The more even skeletal distribution of the isotope following chronic ingestion results in a relatively greater dose rate to the periosteum than following injection, but evidently not great enough to kill the cells that have the potential of becoming neoplastic.

The pathogenesis of the ^{90}Sr effects on hematopoiesis must be very complex with this long-lived, bone-seeking radionuclide, since the delicate homeostatic balance between stimulatory and repressor feed-back mechanisms would be under constant perturbation from the continuous marrow damage. In addition to this direct radiation damage, the cells responsible for maintaining the microcirculation are probably injured and contribute to the hematopoietic cell changes. It has, in fact, been shown by Crosby and co-workers[19] that, in X-irradiated animals, delayed bone marrow aplasia from the deterioration of the microcirculation results in the hematopoietic cells not being maintained. It is reasonable to presume that a similar situation could ultimately occur at higher ^{90}Sr dose levels, and could be primarily responsible for the terminal fall in peripheral blood elements, and contribute to the subsequent development of myeloid metaplasia.

It is apparent that continuous ingestion of ^{90}Sr, exclusive of the 125 μCi/day group, results in either a rather severe hematodepression and a terminal hemorrhagic crisis (3100, 650, or 250 μCi/day), or has no readily detectable effect on the formed blood elements (< 125 μCi/day). The leukemogenicity, especially at the 125 μCi/day level, is particularly interesting and is in contrast to earlier studies in which ^{90}Sr by injection indicated that bone tumor production would be the most prevalent neoplasm encountered. This observation confirms the results of retrospective studies in the human by Vaughan,[14] in that she found the latent period for leukemia induction was considerably shorter than that for osteosarcoma development.

The sequence of events terminating in acute hematopoietic neoplasms in these pigs is unknown. There are, of course, several possible mechanisms which may act independently or in concert. The effects of chronic leukopenia would undoubtedly influence the humoral feed-back control mechanisms, presumably by elevating "leukopoietin" levels. An elevated "poietin" level probably would also be related to a reduced chalone effect if early cell death occurred. There is some evidence from the bone marrow examinations of the uterine-milk study pigs that this may be occurring at the higher ^{90}Sr

levels. The increase in blast and pro forms could be either a quantitative increase in the cell types or a relative increase if more distal cells have died. Also, Cooper et al.[20] demonstrated a prolonged granulocyte generation time in six pigs ingesting 625 μCi ^{90}Sr/day. Beirman[21] reports similar findings in human acute leukemia patients, along with defective maturation and altered release of cells to the peripheral blood. These effects would certainly upset the feedback control mechanisms.

An impaired immunologic competence, with either a viral or neoplastic clonal etiology, must also be considered in the development of these hematopoietic neoplasms and is under investigation at this time. Viruses with certain characteristics common to known leukemia viruses have been isolated from some of these leukemic pigs, but their role in the etiology is unknown.

A scheme depicting the possible pathogenesis of ^{90}Sr induced hematopoietic neoplasms is shown in Figure 5. Bone marrow irradiation may produce some subtle somatic mutation, resulting in an enzymatic change and a maturation defect. This clone of cells could then become the dominate cell line, by either progressive radiation effects on the normal cell line, or by a failing immune system which had previously held the neoplastic cells in check. Probably both mechanisms would be active. In addition, this clone of immature cells might be especially susceptible to the cellular and humoral regulators that would be elevated from the constant marrow damage. It has been suggested that the spectrum of hematopoietic neoplasms in these pigs indicates that a pluripotent marrow stem cell is probably affected.[13,14] This, however, does not seem reasonable since most neoplasms have been of a single cell type, which would indicate the affected cell is probably a committed stem cell. It should be noted from Figure 5 that myeloid metaplasia is not a part of the progression to neoplasia, but is probably a physiologic response to the pathologic process occurring in the bone marrow.

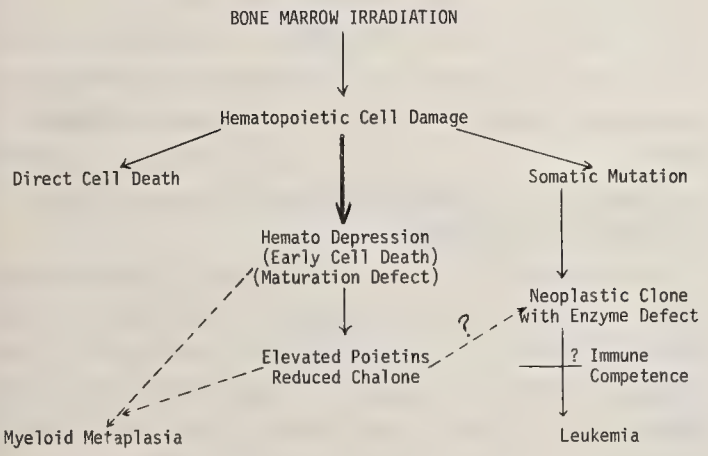


Figure 5

Proposed Scheme of ^{90}Sr Induced
Myeloid Metaplasia and Neoplasia

FIGURE 5.—Proposed Scheme of ^{90}Sr Induced Myeloid Metaplasia and Neoplasia.

SUMMARY

The "gross" effects of ^{90}Sr have been well documented in several species. However, there needs to be additional studies of in-utero and neonatal exposure of the hematopoietic system, correlated with the subsequent development of hematopoietic neoplasia. These more definitive studies would include determination of stem cell kinetics, the influence of poietins and chalones, and alterations in leukocyte kinetics. These questions need answers not only for clarification of cellular radiation effects, but also because non-radiation bone marrow insults may have a similar pathogenesis in the development of neoplasia. The miniature pig is particularly well suited for these studies because of life span and the ease of obtaining serial blood and bone marrow samples.

ACKNOWLEDGMENTS

The contributions of L. K. Bustad, R. O. McClellan, M. E. Kerr, and many others to the early periods of this study are gratefully acknowledged.

REFERENCES

1. PARMLEY, W. W., JENSEN, J. B., and MAYS, C. W. Skeletal self-absorption of beta-particle energy. In: *Some Aspects of Internal Radiation*, T. F. Dougherty, W. S. S. Jee, C. W. Mays, and B. J. Stover (Eds.), Permagon Press, Oxford, pp. 437–451, 1962.

2. PALMER, R. F., THOMAS, J. M., WATSON, C. R., and BEAMER, J. L. Some aspects of dosimetry in miniature swine chronically ingesting ^{90}Sr. *Health Phys.* 19:775–783, 1970.
3. HORSTMAN, V. G., CLARKE, W. J., HACKETT, P. L., KERR, M. E., PERSING, R. L., and BUSTAD, L. K. Anatomic and physiologic data in miniature swine. In: *Hanford Atomic Products Operation Report HW-65500:59–67*, 1960.
4. MCCLELLAN, R. O., CLARKE, W. J., MCKINNEY, J. R., and BUSTAD, L. K. Preliminary observations on the biologic effect of ^{90}Sr in miniature swine. *Am. J. Vet. Res.* 23:910–912, 1962.
5. MCCLELLAN, R. O., VOGT, G. S., and RAGAN, H. A. Age-related changes in hematologic and serum biochemistry parameters in miniature swine. In: *Swine in Biomedical Research*, L. K. Bustad and R. O. McClellan (Eds.), Frayn Printing Co., Seattle, pp. 597–610, 1966.
6. MOORHEAD, P. S., NOWELL, P. C., MELLMAN, W. J., BATTIPS, D. M., and HUNGERFORD, D. A. Chromosome preparation of leukocytes cultured from human perpheral blood. *Exp. Cell Res.* 20:613–616, 1970.
7. CLARKE, W. J., PALMER, R. F., HOWARD, E. B., and HACKETT, P. L. Strontium-90: Effects of Chronic ingestion on farrowing performance of miniature swine. *Sci.* 169:598–600, 1970.
8. *Recommendations of the International Commission on Radiological Protection*, ICRP Publication 9, Permagon Press, New York, 1966.
9. *Report of U.N. Scientific Committee on Effects of Atomic Radiation*, Geneva Assembly Official Records, 19th Session, Supplement 14 (A-5814). United Nations, New York, 1964.
10. CLARKE, W. J., HACKETT, P. L., and RAGAN, H. A. Radiostrontium chronic toxicity study. *Pacific Northwest Laboratory Annual Report for 1970*, to U.S. Atomic Energy Commission, BNWL-1550, p. 46–53, 1971.
11. RAGAN, H. A., WATSON, C. R., GILLIS, M. F., BEAMER, J. L., CLARKE, W. J., HOWARD, E. B., HACKETT, P. L., VOGT, G. S., and PALOTAY, J. L. Effects of radiostrontium in miniature swine, 7th progress report. *Pacific Northwest Laboratory Report* to the U.S. Atomic Energy Commission for 1966, BNW-480, p. 23–27, 1967.
12. HOWARD, E. B., CLARKE, W. J., KARAGIANES, M. T., and PALMER, R. F. Strontium-90 induced bone tumors in miniature swine. Rad. Res. 39:594–607, 1969.
13. HOWARD, E. B., and CLARKE, W. J. Induction of hematopoietic neoplasms in miniature swine by chronic feeding of strontium-90. *J. Natl. Canc. Inst.* 44:21–38, 1970.
14. VAUGHAN, JANET. Radiation in myeloproliferative disorders in man. In: *Myeloproliferative Disorders of Animals and Man*, W. J. Clarke, E. B. Howard and P. L. Hackett (Eds.), U.S. Atomic Energy Commission Division of Technical Information, CONF-680529, p. 489–500, 1970.
15. FINKEL, M. P. and BISKIS, B. O. Experimental induction of osteosarcomas. In: *Progress Exptl. Tumor Res.*, F. Homburger (Ed.), Hafner, N.Y. Vol. 10, pp. 72–111, 1968.
16. MAYS, C. W., DOUGHERTY, T. F., TAYLOR, G. N., LLOYD, R. D., STOVER, B. J., JEE, W. S. S., CHRISTENSEN, W. R., DOUGHERTY, J. H., and ATHERTON, D. R. Radiation induced bone cancer in beagles. In: *Delayed Effects of Bone-Seeking Radionuclides*, C. W. Mays, et al. (Eds.), Univ. of Utah Press, Salt Lake City, pp. 387–408, 1969.
17. MOLE, R. H. Endosteal sensitivity to tumor induction by radiation in different species: A partial answer to an unsolved question. In: *Delayed Effects of Bone Seeking Radionuclides*, C. W. Mays et al. (Eds.), University of Utah Press, Salt Lake City, pp. 249–261, 1969.
18. CLARKE, W. J. Comparative histopathology of ^{239}Pu, ^{226}Ra, and ^{90}Sr in pig bone. Health Phys. 8:621–627, 1962.
19. CROSBY, W. H. Experience with injured and implanted bone marrow: Relation of function to structure. In: *Hematopoietic Cellular Proliferation*, F. Stohlman (Ed.), Grune and Stratton, New York, pp. 87–96, 1970.
20. COOPER, P. H., and CLARKE, W. J. Marrow hematopoiesis in Pitman-Moore swine following ^{90}Sr ingestion. In: *Myeloproliferative Disorders of Animals and Man*, W. J. Clarke, E. B. Howard, and P. L. Hackett (Eds.), USAEC Tech. Inf. CONF-680529, pp. 71–83, 1970.
21. BIERMAN, H. R. The leukemias—proliferative or accumulative? Blood 30:238–250, 1967.

DISCUSSION

CHAIRMAN JOEL: Are there any questions or comments for Dr. Ragan and his presentation? Yes?

FRANCIS L. EARL, Food and Drug Administration: (Inaudible)

DR. RAGAN: No, I didn't give an explanation for it. Statistically it was not different, and there are a large number of animals involved in this particular analysis at that dose level. At some of the higher dose levels, there are fewer numbers, but they were not significant.

DR. EARL: But, on your slide, you infiltrate them. Is this a leukocytic infiltration that makes this appearance so malignant?

DR. RAGAN: No. It depends on the cell types of our leukemia. If it's a granulocytic leukemia, we get that infiltrated with the neoplastic gran-

ulocytes. If it's lymphocytic, of course, then it's a lymphocytic infiltration.

DR. EARL: Have you done any comparisons?

DR. RAGAN: You mean feeding?

DR. EARL: I'm particularly interested in bone marrow effects.

DR. RAGAN: No, Marv Goldman might be able to, he could answer the part on the dogs.

DR. EARL: The question I'm really after is that I'm of the opinion that insults to the bone marrow on the pig are more serious than insults to the bone marrow of dogs, and I'm wondering if other people have found this to be true.

DR. RAGAN: Dr. Goldman?

MARVIN GOLDMAN, University of California, Davis: We had somewhat a similar finding from several hundred beagle dogs.

DR. RAGAN: I think it is interesting that, although lymphatic leukemia or lymphosarcoma is supposedly the most commonly observed in swine, we have seen no cases in our control animals. Our two cases in the controls have been granulocytic.

J. W. CLAYTON, University of Wisconsin: First of all, I take the opportunity to congratulate you on a very elegant design. My question has to do with the toxicological aspects of when you recorded, in the sense that the percentage of stillbirths was around 5%, and both (I think there were 25 and 5 in the two groups) were about the same. I wonder if the fetuses were examined for any teratogenic effects, other than skeletal deformity?

DR. RAGAN: No. They were not. These were just gross observations.

DR. CLAYTON: My next question relates to the conduct of the "no effect" level. It would appear from your data that the dose was below 25 microrads. Could you comment on that?

DR. RAGAN: A "no effect"? I don't know that I believe that there is a "no effect" level. We are not able to show it with the life span of these animals at the doses we're using, with these rather gross assays. Now I'm certain that with more sophisticated techniques that differences (effects) can be shown, but this was essentially an "effect-no effect" type of study.

DR. CLAYTON: How about the idea that the F1's and F2's within two generations had a slightly higher number of tumors of one type than the parents did, even though the dose was in about the same range?

DR. RAGAN: The F1's and F2's have a slightly higher dose. They are, of course, exposed from in utero on.

DR. CLAYTON: Yes, but how would you explain the context of dosage—all dosage as the rate of dosage or some other idea of dosage?

DR. RAGAN: Are you speaking of accumulated dose or a dose rate? I think probably the dose rate is also important in this.

DR. CLAYTON: I'm not sure whether it was in the next to the last slide, the average rad dose to parents was about 10 to 20 and to offspring it was about 13 to 17; it would be about the same dosage as far as accumulated dose was concerned.

DR. RAGAN: The time of appearance of these neoplasms in the F1–F2 has a mean of about 36 months. Whereas, in the originals, it's up about 70, which I think again points to a possible feedback mechanism in which these animals, the F1's and F2's, are more severely involved. Also, their homeostatic mechanisms are probably turned on a little more.

A FOUR-YEAR STUDY OF LONG-TERM EFFECTS OF IMPLANTED DISCRETE HEAT AND RADIATION SOURCES IN DOGS AND PRIMATES: PRELIMINARY STUDIES LEADING TO IMPLANTABLE NUCLEAR-FUELED CIRCULATORY SUPPORT SYSTEMS

G. W. Sandberg, Jr., F. A. Molokhia,
P. R. Ponn, F. N. Huffman and J. C. Norman**

Recipients of totally implanted nuclear powered circulatory support systems will be exposed continually to intracorporeal heat and radiation. Physiologic effects of thermal and nuclear radiation doses expected from clinical systems are being investigated using canine and primate models. Plutonium 238-fueled heat exchangers were implanted in series with the aortas of large dogs; chronic preparations have been obtained and effects of the thermal, gamma, and neutron radiation studied. Radiation equivalent sources (RES) composed of Americium 241-Beryllium/^{90}Strontium with equivalent photon and neutron dose rates but without thermal outputs were implanted in dogs and primates; chronic effects have been studied. A dog with a 16 watt ^{238}Pu heat exchanger in the abdominal aorta survived 25 months before dying from unrelated causes; and another mongrel lived for 26 months with a 24 watt thoracic aortic implant. Serial blood studies showed no significant abnormality, and at post-mortem, microscopic changes were only seen within 1 cm of the radiation source. These and similar results from chronic RES implants suggest that there are no compelling biologic reasons to prevent eventual application of totally implanted nuclear power sources for artificial circulatory support systems.

INTRODUCTION

Research leading to the development of nuclear-fueled thermal power sources [1] for totally implantable cardiac support and replacement devices has been in progress in our laboratories for the last six years. An integrated system will contain a radioisotope heat source, a thermal engine [2,3] capable of supplying power for blood pumping [4] to a circulatory assist [5] or cardiac replacement pump, as well as the necessary control logic package.

The recipient of such a nuclear-powered device will be exposed to continuous intracorporeal thermal and nuclear radiation. Preliminary investigation suggested that intracorporeal thermal loads up to 0.64 watts/kg are well tolerated in the acute experimental situation.[6,7] In order to determine if an animal can tolerate chronic thermal and nuclear radiation levels of the magnitude expected from clinically implanted nuclear power sources for artificial circulatory support systems, animal model studies using implanted radioisotope capsules have been carried out.

Animals were studied after the implantation of one of three kinds of radioisotope capsules: (1) Canine aortic heat exchangers fueled with Plutonium-238 (^{238}Pu) capsules with thermal outputs of 16 to 24 watts; (1) Canine thoracic or iliac fossa implants of "Radiation Equivalent Source" capsules (RES) which have radiation outputs similar to 24 watt ^{238}Pu capsules, without significant thermal output; and (3) Primate iliac fossa implants of "RES–50" capsules with radiation characteristics similar to 50 watt ^{238}Pu sources. The sections which follow describe each model and summarize the results obtained from the studies.

^{238}Pu-FUELED AORTIC HEAT EXCHANGER MODEL

Plutonium-238 is the nuclear fuel most likely to be utilized as a heat source in implantable thermal engines which can power artificial circulatory support devices.[8] Already, the isotope is used in the nuclear powered pacemakers being studied in the U.S., England, and France.[9] Plutonium 238 is an artificial radioisotope pro-

* Present address: Medical Devices Applications Program, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland.

** Sears Surgical Research Laboratory, Harvard Medical School, Boston City Hospital, Boston, Massachusetts; and Thermo Electron Corporation, Waltham, Massachusetts.

duced as a by-product of uranium-fueled reactors. It decays by alpha particle and gamma ray emission to ^{234}U with a half-life of 85 years, and it also undergoes spontaneous nuclear fission with a half-life of 4.9×10^{10} years. The alpha decay results in the production of heat as the high energy heavy alpha particles are decellerated. The thermal power density of ^{238}Pu in the metallic form is 0.55 watts/gram or 32.1 watts/kilocurie, making it a suitable implantable heat source. Plutonium is not only a source of heat, but also a source of potentially dangerous neutrons and photons. These are from the following six sources:[10] (a) Neutrons from spontaneous fission; (b) Neutrons produced by alpha particle reactions (α,n) with low atomic number impurities; (c) Fast fission neutrons; (d) Photons from ^{238}Pu decay; (e) Photons and neutrons from other plutonium isotope contaminants and their daughters; and (f) Photons produced by alpha particle (α,γ) reactions with light impurities.

In order to permit study of the biological effects of chronic intracorporeal exposure to ^{238}Pu, an implantable aortic heat exchanger was designed. An insulated solid aluminum carrier containing the radioisotope capsule is attached to a metallic tube which is interposed in place of an excised segment of either thoracic or abdominal aorta.[11] (Figure 1) Heat from the fuel capsule is conducted by the carrier to the blood which carries it away to be dissipated by the recipient's thermoregulatory mechanisms. The recipient is also exposed to neutron and gamma radiation. Total tissue neutron plus gamma dose rates fall off from the order of 10^3 mrem/hr at the source surfaces to 20.0 and 34.3 mrem/hr 8cm from the 16 and 24 watt capsules respectively.

Eleven dogs were implanted with heat exchangers fueled with either 16 watt or 24 watt ^{238}Pu capsules. At maximum, this represented a thermal dose of 0.4 watts/kg. The acute thermoregulatory responses of all animals to the heat load were observed. A long-term survivor of implantation of both the 16 and 24 watt cap-

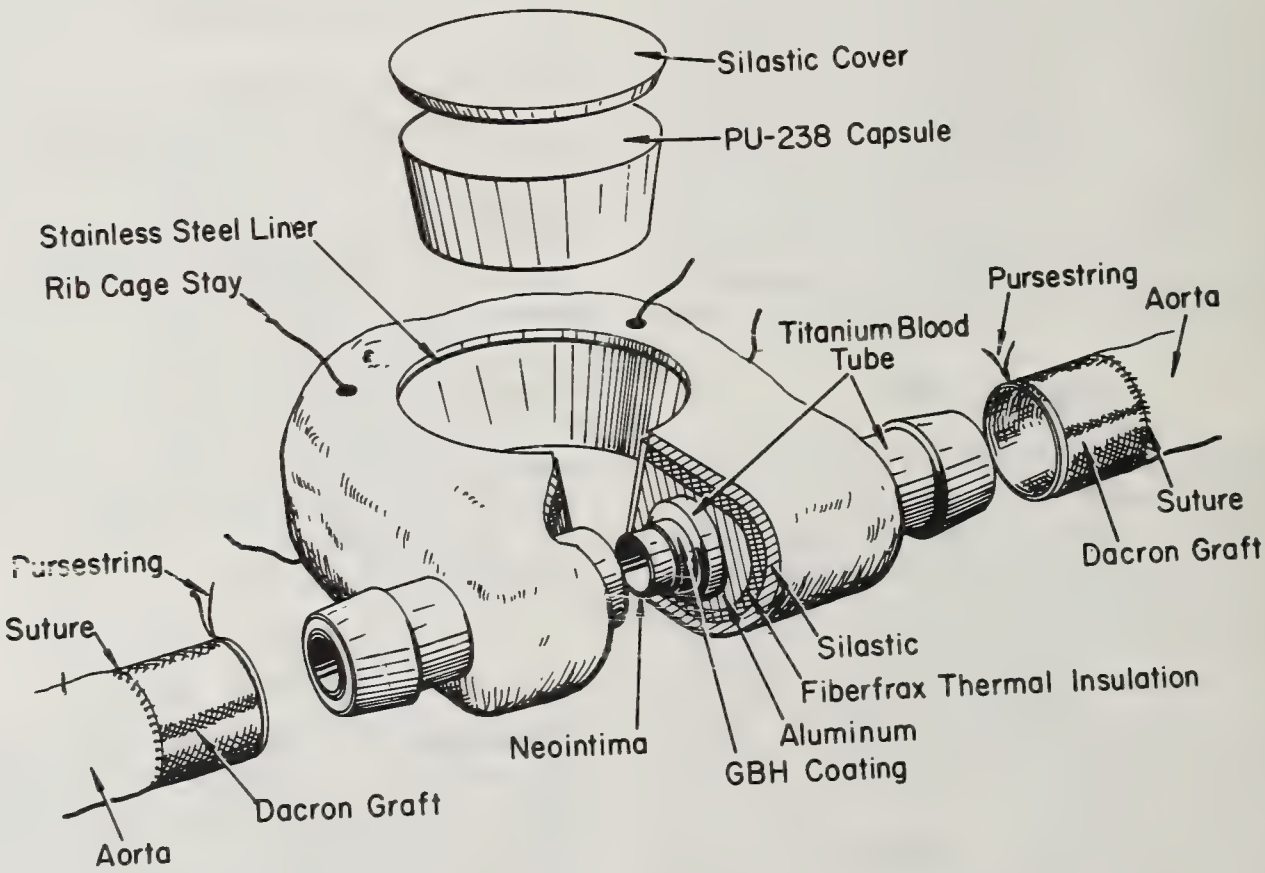


FIGURE 1.—Drawing showing design of ^{238}Pu-fueled heat exchanger which is interposed in either the thoracic or abdominal canine aorta.

sules was observed for physiologic and pathologic effects; the 16 watt recipient survived 25 months and the 24 watt recipient lived 26 months.

The rectal temperatures of all animals increased immediately after heat source implantation from one to 2.5 degrees centigrade, but within 24–72 hours, the temperatures had all returned to normal. (Recipients of the 24 watt capsule required external body cooling until the effects of anesthesia had disappeared.) Thereafter, the animals maintained normal rectal temperatures in spite of the intracorporeal heat load. This indicates that an animal can dissipate, both in the acute phase and chronically, a thermal load of the magnitude expected from a thermal artificial heart power source.

The two chronic survivors were monitored for evidence of adverse effects of the heat and radiation. Serial observation, physical examinations, and repeated batteries of blood studies revealed no evidence of significant abnormality of neurological, cardiovascular, gastrointestinal, hepatic, renal, endocrine, or hematologic function. Serial studies of lymphocyte cultures for chromosomal aberration as a reflection of radiation effect were conducted; results of these studies can be found elsewhere in these *Proceedings.*

The recipient of the 16 watt capsule died of pulmonary embolism 25 months after implantation; the cause of the thromboembolus which came from a pelvic vein could not be related to the chronic heat or radiation exposure. The recipient of the 24 watt capsule was in good condition when it was sacrificed after 26 months. At the time of sacrifice, blood temperatures were measured[12] within the heat exchanger tube. The maximum temperature was 45.4°C; the temperature of the blood leaving the exchanger was 40.0°C while body temperature and that of the blood entering the device was 38.6°C. In spite of these locally elevated blood temperatures no damage to the animal's formed blood elements, plasma proteins or coagulation factors could be documented in serial samples of peripheral blood. Cardiac catheterization at time of sacrifice revealed normal hemodynamic parameters with the exception of marginally elevated pulmonary arterial pressure (30 torr) after isoproterenol infusion. No structural etiology for this could be demonstrated at necropsy.

An extensive gross and microscopic postmortem examination was made of both long-term animals; effects of the chronic heat and radiation were specifically sought. Significant pathology was limited in both animals to the tissue which was located within one centimeter of the radiation source. In this tissue were seen necrosis, fibrosis, inflammation and rarely, fibrocytes which showed some evidence of radiation effect. Lung tissue at about 1 cm from the source revealed mild radiation changes. In addition, there was marrow depletion in a rib overlying the source which was probably the result of radiation exposure. This tissue had been exposed to approximately 1000 mrem/hr for 26 months. Beyond 1 cm, there was no apparent evidence of radiation damage; no neoplastic changes were observed.

CANINE RADIATION EQUIVALENT SOURCE (RES) MODEL

The ^{238}Pu capsules exposed the recipients to both heat and nuclear radiation. In an attempt to separate their effects, the RES capsule was designed to have neutron and photon outputs similar to ^{238}Pu capsules but without thermal emission.[13] A mixture of Americium-241 and Beryllium was selected as the neutron source and Strontium 90 as the source of photons. Alpha particles emitted by the decaying ^{241}Am interact with Be nuclei, and neutrons are emitted by the (α,n) reaction. Photons are produced by the Bremsstrahlung effect as β^- emitted by the ^{90}Sr are decellerated by a graphite scatterer. The total radiation output of the RES capsule is similar to that of the 24 watt ^{238}Pu source; the total tissue dose rate at the capsule surface is aproximately 2000 mrem/hr.

Silastic encapsulated RES capsules have been implanted in the thoracic cavities or iliac fossae of six dogs. (Figure 2) Two (a male and a female) are alive more than 50 months after implantation; they have produced 3 litters of offspring. Another is alive 1½ years after implantation. Three died or were sacrificed at 3, 5, and 14 months post-implantation. The

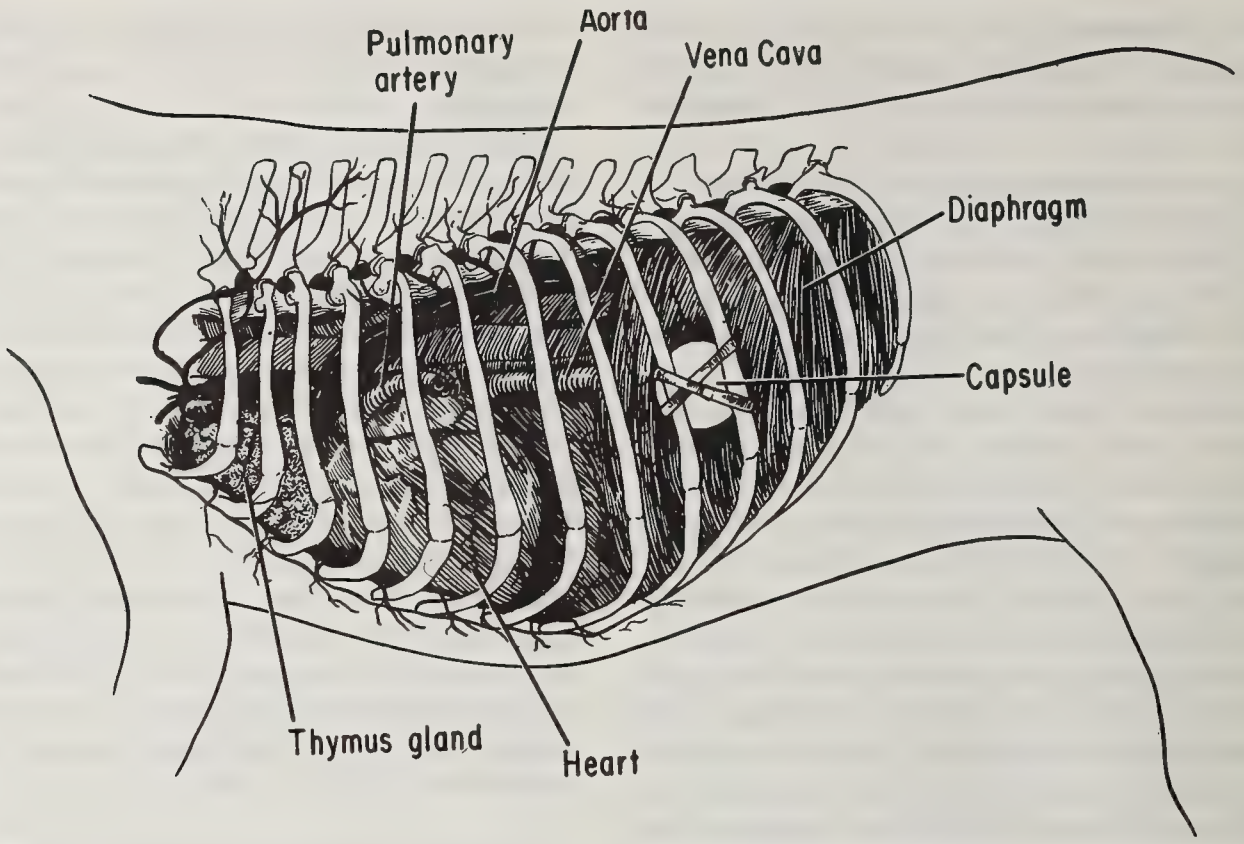


FIGURE 2.—Anatomic relations of Radiation Equivalent Source capsule implanted in the left costophrenic sulcus of a dog.

long-term survivors have been studied with serial blood studies including chromosome analysis; unequivocal changes reflecting radiation effects have not been observed. Studies of offspring of RES-bearing parents have not revealed evidence of radiation damage although post natal survival rates for the first 2 litters were low, probably due largely to environmental factors. These offsprings resulted from gametes produced by radiated parents, and the pups developed *in utero* exposed to 5–170 mrem/hr total radiation.

The three animals which died were studied pathologically. One died at three months of pneumonia and had no evidence of radiation exposure. A second recipient had been exposed to 5 months of RES radiation before dying of wound infection. Although infection and foreign body reaction complicated the histopathological picture, some evidence of radiation-induced bone marrow suppression in a rib just adjacent to the capsule site was found. The most valuable pathological study was that of an animal sacrificed 14 months post RES implantation.[14,15] Detailed histopathology revealed no abnormalities attributable to intracorporeal radiation except within 6 mm of the RES capsule. In this area, which had been exposed to approximately 2000 mrem/hr total radiation for 14 months, the following changes were seen: Necrosis with a halo of leucocytic infiltration, abnormal radiation fibrocytes, excess fibrosis and hyalinization, microvascular abnormalities including abnormal endothelial cells, endothelial proliferation and telangiectasis, and abnormal pulmonary alveolar cells and alveolar linings. (Figure 3) These changes represented more severe evidence of radiation effect than seen in the areas adjacent to the 24 watt ^{238}Pu source. The latter tissue has been exposed to a somewhat lower radiation dose rate than the former, but for 26 instead of 14 months.

PRIMATE RES-50 MODEL

The RES–50 radioisotope capsule was designed to produce neutron and photon dose rates roughly equivalent to a 50 watt ^{238}Pu

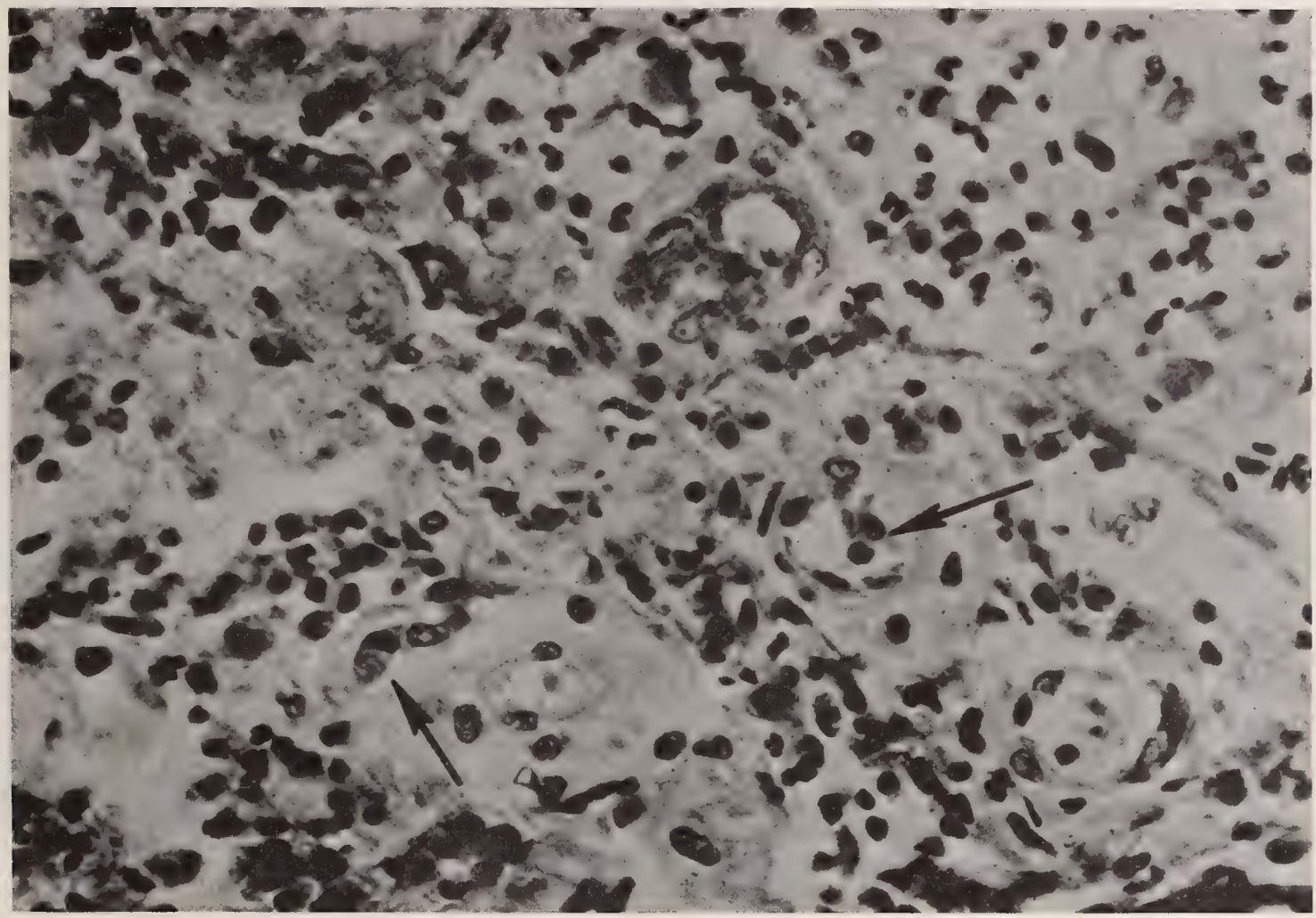


FIGURE 3.—Photomicrograph (500X) of tissue within 1 cm of Radiation Equivalent Source capsule after 14 months, showing probable radiation effects. Note abnormal fibrocyte (arrow at left) and abnormal endothelium (arrow at right).

power source capsule, the largest likely to find clinical application. Two ^{241}Am/Be neutron sources, each similar to that in the canine RES are used in the RES–50, together with a ^{90}Sr Bremsstrahlung source of appropriately greater size. RES–50 capsules were implanted in the iliac fossae of two baboons and one chimpanzee. (Figure 4) Monitoring in the 10 months since implantation has failed to reveal systemic evidence of radiation effect. Extensive gross and histopathological studies to characterize tissue response to the elevated radiation dose rates are planned after the period of chronic observation.

SUMMARY

Continuing animal model studies of the biological effects of chronic intracorporeal heat and radiation of the magnitude to be obtained from totally implantable nuclear power sources for circulatory support devices have been described. The acute and chronic responses of dogs to implanted ^{238}Pu-fueled aortic heat exchangers have been studied; canines and primates have been studied after implantation of heatless "Radiation Equivalent Sources" of neutrons and photons. Major observations from these studies can be summarized:

(1) Animals appear to be able to tolerate additional endogenous heat loads of up to 0.4 watts/kg both in the acute adjustment and chronic exposure periods. Local intra-aortic blood temperatures of 45° to 46°C are not incompatible with long-term survival.

(2) Dogs appear to be able to tolerate for more than 3 years continuous intracorporeal



FIGURE 4.—Frontal and lateral X-rays of primate showing RES–50 capsule *in situ* in the left iliac fossa.

neutron and photon radiation of circulatory device power source magnitude without gross ill effect. Such radiation does not preclude successful reproduction.

(3) Histopathological expression of the response to the radiation appears to be limited to the tissue within 1 cm of the radiation capsule after up to 26 months of continuous exposure. Changes which are seen appear mild and do not interfere with any normal physiological function. No neoplastic changes have been observed.

It should be emphasized that the radiation sources studied were fabricated and implanted without radiation shielding. The tissue dose rates produced by these sources are at least three times greater than what realistically can be expected from eventual power source capsules, in which greater ^{238}Pu purity, improved geometry, and radiation shielding will attenuate tissue dose rates.

These preliminary animal model studies are by no means conclusive. Although there appear to be no reasons to suspect that the biologic response of canine tissue to thermal and nuclear radiation is fundamentally different than the human tissue response, the possibility does exist. It is also clear that in order to define the biologic sequellae of chronic simultaneous exposure to heat and radiation, many more animals would have to be studied for longer periods of time than are included in our experience. These studies are encouraging, however, and the data lend support to the notion that eventual application of totally implanted nuclear power sources for artificial cardiac support systems is feasible.

REFERENCES

1. Section XII: Implantable energy systems, in Artificial Heart Program Conference. Ed. R. J. Hegyeli, U.S. Government Printing Office, Washington, pp. 913–1109, 1969.
2. HARVEY, R. J., ROBINSON, T. C., BERNHARD, W. F., NORMAN, J. C., VANSOMEREN, L., LAFARGE, C. G.

Evaluation of the Rankine Steam Cycle as a Power Source for a portable artificial heart. Ed. L. J. Fogel *Progress in Biomedical Engineering*. Spartan Books, Washington, p. 259, 1967.

3. Huffman, F. N., Bornhorst, W. J., Harmison, L. T. A radioisotope fueled vapor cycle power supply for an artificial heart. Trans. Am. Nuclear Soc. 13:503, 1970.
4. Robinson, T. C., Harvey, R. J., LaFarge, C. G., Bernhard, W. F., vanSomeren, L., Norman, J. C. Determination of Blood Pumping Power Requirements for an Artificial Heart, in Fogel, *op. cit*, p. 243.
5. Norman, J. C., Molokhia, F. A., Whalen, R. L., Huffman, F. N., and Harmison, L. T. An advanced left ventricle-to-aorta assist pump compatible with an implantable nuclear power source. Ann. Surg. (in press).
6. Norman, J., LaFarge, G., Harvey, R., Robinson, T., Bernhard, W. Experimental model for inducing acute and chronic hyperthermia: Developmental methods and preliminary observations as related to power requirements of cardiac prostheses. Trans. Am. Soc. Artif. Int. Organs. 12:282, 1966.
7. Bernhard, W., Norman, J., Harvey, R., LaFarge, G., Robinson, T., vanSomeren, L. An investigation of induced chronic hyperthermia and *in vivo* heat dissipation. J. Thoracic Cardiovasc. Surg. 52:611, 1966.
8. Cole, D. W., Mott, W. E., Sagan, L. A. Factors relating to the application of radioisotopes to circulatory support systems. Isotop. Radiat. Technol. 7:138, 1969.
9. Norman, J. C., Sandberg, G. W., Huffman, F. N. Implantable nuclear powered cardiac pacemakers. New Eng. J. Med. 283:1203, 1970.
10. Mullins, L. J., Leary, J. A. ^{238}Pu for biomedical applications. Isotop. Radiat. Technol. 7:197, 1969.
11. Norman, J. C., Covelli, V. H., Bernhard, W. F., Spira, J. An implantable nuclear fuel capsule for an artificial heart. Trans. Am. Soc. Artif. Int. Organs 14:204, 1968.
12. Liss, R. H., Huffman, F. N., Warren, S., Norman, J. C. Electron microscopy and thermal analysis of neointima heated and irradiated *in vivo* for two years. Ann. Thor. Surg. 12:251, 1971.
13. Sandberg, G. W., Huffman, F. N., Norman, J. C. Experimental observations of intracorporeal Strontium–90—Americium 241/Beryllium sources simulating radiation fields from nuclear powered artificial hearts. Ann. Thor. Surg. 9:401, 1970.
14. Sandberg, G. W., Huffman, F. N., Norman, J. C. Implantable nuclear power sources for artificial organs: Physiologic monitoring and pathologic effects, I. Trans. Am. Soc. Artif. Int. Organs 16:172, 1970.
15. Sandberg, G. W., Molokhia, F. A., Huffman, F. N., Norman, J. C. Implantable nuclear source for artificial organs: Preliminary pathologic effects, II. Surg. Forum 21:298, 1970.

ANIMAL RADIATION DATA AND THEIR RELEVANCE TO MAN

Harry E. Walburg, Jr.*

The relevance of animal data to human radiobiology is discussed in terms of whole-body exposure to external radiation, late somatic effects, and induction and acceleration of neoplasms. It appears that the implantation of nuclear-powered medical devices in a few nonhuman primates for less than five years is an inadequate test of the radiation risk to man. A reasonable estimate of risk can be made by determining the quality of radiation, its dose-rate, and the dose to the susceptible tissue and applying to these data the entire body of knowledge on radiation carcinogenesis in man and animals.

INTRODUCTION**

The broad topic of the relevance of animal data to human radiobiology has received repeated treatment in the literature over the past 30 years. It will suffice to outline in general terms the conclusions drawn from examinations of experimental animal data without extensive documentation which is readily available in the literature.[1–6] More recent treatments of this topic have been published by Storer and Bond[7] and by the United Nations Scientific Committee on the Effects of Atomic Radiation.[8,9]

In addition to limiting the amount of documentation, I will restrict the discussion to the following: (1) whole-body exposure to external radiation, where there is a relatively uniform dose to all cells of the body, permitting an accurate determination of the dose received by the susceptible tissue or cell populations, (2) late somatic effects, excluding genetic effects and the hematologic disorders seen within approximately 90 days following irradiation, and (3) induction and acceleration of neoplasms, which is the most significant late effect following exposure to low-level radiation.

* Biology Division, Oak Ridge National Laboratory, Oak Ridge. Tennessee 37830.

** Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

METHODS

One of the principal problems we face in extrapolating experimental data to man is the variability in type and sensitivity of tumors induced by radiation in different species and even among different strains of the same species. For example, it is well known that mice are highly susceptible to the induction of certain types of hemopoietic neoplasms (principally thymic lymphoma), as well as female endocrine organ tumors (i.e., ovarian, pituitary, and breast).[5] Swine, on the other hand, are susceptible to induction of liver, intestinal, and uterine tumors,[10] while burros show no evidence of tumor induction by whole-body radiation.[11] Man appears to be most susceptible to induction of certain forms of leukemia (i.e., acute and chronic myelogenous and acute lymphatic) and thyroid carcinoma.[9] Such variation is common even among strains of the same species. While mice of the RFM strain are highly susceptible to the induction of thymic lymphoma and myeloid leukemia, C3H mice rarely develop these diseases following whole-body radiation, and C57BL mice are intermediate between the other two strains in incidence of thymic lymphoma but do not develop myeloid leukemia (H. E. Walburg, Jr., unpublished data). With such variation evident, it seems highly unlikely that a single adequate model exists which will permit direct quantitative extrapolation to man of experimental data on radiation carcinogenesis.

How, then, can such experimental data be useful in estimating man's risk to neoplasia from radiation? In general there are three possibilities, as previously outlined by Mole:[12] (1) directly applicable models, (2) useful qualitative generalizations, and (3) useful quantitative generalizations. While directly applicable mod-

els have not been documented, there are some qualitative observations which appear to be independent of species and genetic or environmental influences. These are outlined below. An example of a useful quantitative generalization would be the relation of cell replication rate to the probability of tumor induction following irradiation of a given tissue. If this relationship were independent of strain and species, then determination of cell replication rates in human tissue could be used to predict tumor incidence in man for that tissue. While no such quantitative generalizations are obvious at present, their potential importance is sufficient to justify a continuing search, for as the mechanisms of radiation carcinogenesis are clarified, quantitative generalizations may also be reached.

The useful qualitative generalizations drawn from experimental radiation data can be summarized as follows.[8]

1. Radiation shortens the mean life-span.
2. Such life-shortening can be explained as principally due to induction and acceleration of neoplastic diseases, especially for low doses and low dose rates of radiation.
3. Dose-response curves for the induction of neoplasms are characterized by an increase in incidence with increasing dose up to a maximum, followed by a decline as the dose continues to increase. Where a tumor system is easily induced by small amounts of radiation (e.g., thymic lymphoma in mice, breast tumors in rats), an absolute threshold (i.e., a dose below which there is no effect) has not been demonstrated due to the large numbers of animals required for statistical proof; but for more resistant tumor systems (e.g., kidney tumors induced by local organ irradiation in rats, skin tumors by local irradiation in rats) an absolute threshold seems to be established.
4. High-LET radiation (e.g., fission neutrons) is more effective for a given dose than low-LET radiation (e.g., gamma rays).
5. For the same total accumulated dose, high dose rates are more effective than low dose rates.
6. Low-LET radiation shows a more pronounced dose-rate effect than high-LET radiation.
7. Juvenile animals are generally more susceptible than adults to the induction of neoplasms.

If these generalizations are indeed independent of species, then they should be applicable to irradiated human populations. The irradiated human population most similar to the experimental populations discussed above is the Japanese atom-bomb survivors. Contrary to most other human populations studied, these individuals were not selected on the basis of illness or a single parameter such as occupation, and they received whole-body rather than localized irradiation. The qualitative generalizations drawn from animal data apply to this population as follows.[9]

1. Radiation does shorten the life-span.
2. This life-shortening can be explained on the basis of induced or accelerated neoplasia.
3. The dose-response curve for leukemias is similar to the curves seen with the experimental animal data.
4. Differences in the induction of leukemia between the irradiated populations of Hiroshima and Nagasaki can be explained on the basis of increased efficiency of the radiation from the bomb exploded at Hiroshima, radiation which contained a larger component of neutrons than was received by the Nagasaki population.

5,6. There are no dose-rate data available for this population.

7. Young members of the irradiated population appear to be more sensitive to the induction of thyroid carcinoma than their adult counterparts.

Thus, in human populations exposed to whole-body radiation there do not appear to be any peculiar responses that would invalidate the qualitative generalizations developed from experimental animal data.

In general, evaluation of the scanty data from irradiated human populations in the light of the

qualitative generalizations outlined above has resulted in the best estimates of risks to man from radiation. Turning to the specific problem of estimating the risk to man of radiation emanating from implanted nuclear-powered medical devices, several points must be emphasized.

1. There is no evidence that data for late somatic effects of radiation from any single animal model system, not even the nonhuman primates, can be extrapolated quantitatively to man.
2. Since we are dealing with low levels of radiation, which result in small (but not necessarily unimportant) risks of induction of neoplastic diseases, large numbers of animals are required to validate statistically the level of risk involved.
3. Induction of neoplasia occurs characteristically late in life, requiring studies throughout the entire life-span of the population at risk.

SUMMARY

It seems clear that the risk of inducing or accelerating neoplasms by radiation emanating from implanted nuclear-powered medical devices cannot be determined by implanting these devices (or "radiation-equivalent sources") in a few nonhuman primates and examining them for pathology within two to five years. While these studies may be valuable for determining the amount of "acute" pathology, such as tissue necrosis, aneurism, etc., it is the risk of induction of neoplasms that must be estimated. A reasonable estimate of risk can be made by determining the quality of the radiation, its dose-rate, and the dose to the susceptible tissue and then applying to these data the entire body of knowledge on radiation carcinogenesis in man and animals.

REFERENCES

1. Moskalev, Yu. I., and V. N. Streltsova. The carcinogenic effects of ionizing radiation. A review of current problems. Atomic Energy Review 2(3): 149–194 (1964).
2. Casarett, G. W. Experimental radiation carcinogenesis. Progr. Exp. Tumor Res. 7:49–82 (1965).
3. Van Cleave, C. D. *Late Somatic Effects of Ionizing Radiation*, pp. 78–149. USAEC Division of Technical Information, Oak Ridge (1968).
4. Upton, A. C. Comparative aspects of carcinogenesis by ionizing radiation. Nat. Cancer Inst. Monogr. 14:221–242 (1964).
5. Upton, A. C. Comparative observations on radiation carcinogenesis in man and animals. In *Carcinogenesis: A Broad Critique*, pp. 631–675. Proc. 20th Symp. Fundamental Cancer Research, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, 1966. Williams and Wilkins, Baltimore (1967).
6. Upton, A. C. Radiation carcinogenesis. In *Methods in Cancer Research*. (H. Busch, ed.), vol. 4, pp. 53–82. Academic Press, New York (1968).
7. Storer, J. B., and V. P. Bond. Evaluation of long-term effects of low level whole body external radiation exposures. In *Proc. IV Int. Conf. Peaceful Uses of Atomic Energy, Geneva, Sept. 6–16, 1971*. Unipub, Inc., New York (1972).
8. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). *Experimental Induction of Neoplasms by Radiation*. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, Annex G (1972a).
9. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). *Radiation Carcinogenesis in Man*. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, Annex H (1972b).
10. Brown, D. G., and D. F. Johnson. Clinical and gross pathological findings in swine relative to late effects of mixed gamma-neutron and x-irradiation. Radiat. Res. 44:498–511 (1970).
11. Brown, D. G., D. F. Johnson, and F. H. Cross. Late effects observed in burros surviving external whole-body gamma irradiation. Radiat. Res. 25:574–585 (1965).
12. Mole, R. H. Late effects of exposure to ionizing radiation: animal experimentation and human protection. In *First European Symposium on Late Effects of Radiation* (P. Metalli, ed.), pp. 179–199. Comitato Nazionale Energia Nucleare, Roma (1970).

DISCUSSION

McClellan G. Blair, ARCO Nuclear Co.: I note that your studies have all been of full body radiation. Have you noted the effect of small size or local radiation?

Dr. Walburg: Yes, there is quite a bit of data. Dr. Goldman and others have data on internally deposited radionuclides. I avoided discussing that case because one gets into the

problem of knowing precisely what the dose to the susceptible cell population is. Local body radiation, either from internally deposited radionuclides or from partial body external radiation, causes life shortening which can be explained exclusively on the basis of induction of neoplasms.

FAROUK A. S. MOLOKHIA, Harvard Medical School: What biological endpoints should be used to monitor the effects of radiation emitted by the implanted devices?

DR. WALBURG: I think that unless one is prepared to mount a very large program with sufficient numbers of animals to detect the effect of low doses by measuring the induction of neoplasms, one can't really do much. What I am saying is that the cell doesn't know, and doesn't care, where the radiation comes from, whether outside the body or from within. It's the effect of the radiation on the cell and on the tissue that matters. I think that the endpoints that were described in the preceding paper are very important but they require higher doses of radiation for effect than are required to induce neoplasms. I would predict on the basis of the available data, that radiation which causes cell death or tissue necrosis will also induce neoplasms.

CHAPTER XI

ANIMAL DISEASES

Experimental Viral Valvulitis

Respiratory Diseases of Dogs, Calves, and Sheep

Virus Infections of "Normal" Healthy Animals

Significance of Simian Viruses

Dirofilaria Immitis: A Review of Present Knowledge

Diseases Encountered During the Conditioning of Random Source Dogs and Cats

Congenital Defects of the Cardiovascular System in Dogs

Spontaneous Diseases in Germfree Animals

Resistance of Swine to Bordetella Rhinitis

EXPERIMENTAL VIRAL VALVULITIS

N. P. DePasquale and George E. Burch*

Non-congenital valvular disease is usually attributed to rheumatic fever even in the absence of a history of rheumatic carditis. The willingness of physicians to accept occult or subclinical rheumatic fever as the cause of valvular disease in those patients who do not have a history of rheumatic fever has frustrated the search for etiologic factors other than rheumatic fever in acute and chronic valvular disease. Experimental studies in mice and cynomolgus monkeys have demonstrated that group B_4 coxsackievirus infection may be associated with valvulitis and mural endocarditis in addition to pericarditis and myocarditis. Coxsackievirus B_4 antigen was identified in damaged valve tissue by immunofluorescent technics. In some animals gross and microscopic lesions, which were strikingly similar to those of chronic valvular disease in man, were observed. There is a need to study the role of viruses in the pathogenesis of acute and chronic valvular disease in man.

INTRODUCTION

Viral disease has rarely been implicated as a cause of mural endocarditis or acute and chronic valvulitis, but clinicians and pathologists have long recognized that a variety of viral agents produce pericarditis and/or myocarditis. There is no known mechanism to explain why the pericardium and myocardium should be susceptible to viral diseases while the mural endocardium and valves are immune to viral attack. Physicians have readily accepted the diagnosis of rheumatic valvulitis even in the absence of a history of rheumatic fever, and this has possibly frustrated the search for etiologic alternatives.

During the course of experiments designed to produce acute and chronic viral myocarditis in animals it was found that coxsackievirus group B_4 infection in addition to producing pericarditis and myocarditis was often associated with mural endocarditis and valvulitis.[1,2] The purpose of this paper is to review the results of these experiments and to discuss some of their implications in human disease.

* Tulane University, School of Medicine, Department of Medicine.

MATERIAL AND METHODS

Two groups of experiments, which have been previously reported, were carried out.[1,2]

Group I: A random breed strain of more than 200 HaM/ICR mice were inoculated intraperitoneally with 0.1 ml of fluid containing either 10^3 50% tissue culture infective dose ($TCID_{50}$) or $10^5 TCID_{50}$ of group B_4 coxsackievirus. The viral stock was originally recovered by Kibrick and Benirschke from a 10-day-old infant who died of encephalohepatomyocarditis.[3] The virus was obtained as monkey kidney culture passage strain. Control fluid from monkey kidney culture was also obtained. Virus and control fluid were stored at $-65°C$. The animals were killed 2 to 60 days following inoculation.

Group II: Seven cynomolgus monkeys were inoculated intravenously with 0.3 ml $TCID_{50}$ of the same strain of virus as in the Group I experiments. Two monkeys were inoculated with 0.3 ml of virus-free kidney culture fluid. The experimental animals were killed 51 to 200 days after inoculation and the control animals were killed 51 to 231 days after inoculation.

RESULTS

Group I: Valvular endocarditis was found in 55% and mural endocarditis in 50% of the mice inoculated with group B_4 coxsackievirus. The tricuspid valve was involved in 43%, the mitral valve in 23%, the aortic valve in 10%, and the pulmonic valve in 5% of animals. Mural endocarditis involved the right ventricle in 20%, the right atrium in 18%, the left ventricle in 13%, and the left atrium in 10% of animals.

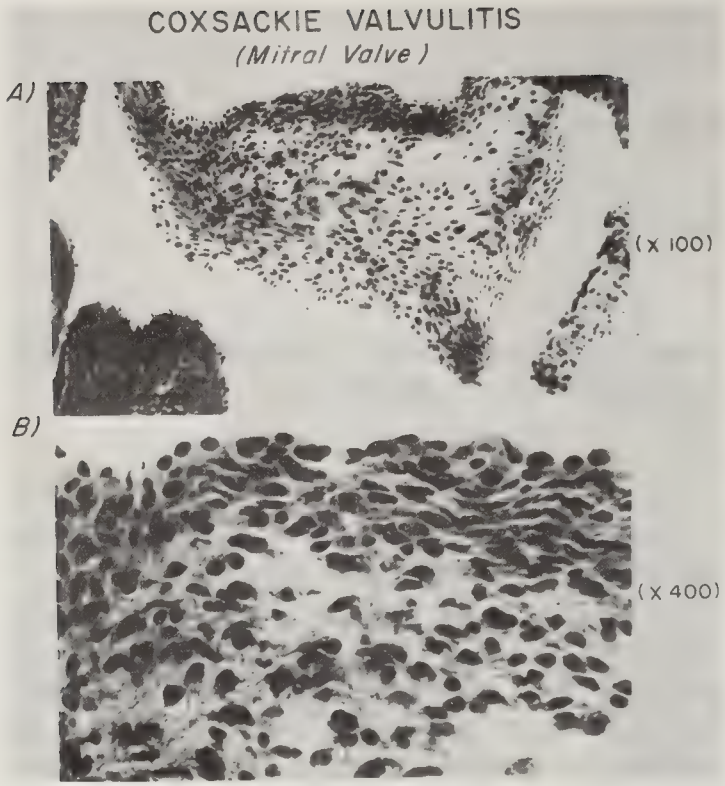


FIGURE 1.—(A) Aortic leaflet of the mitral valve of an 18-day-old mouse killed 5 days after inoculation with coxsackievirus B_4 showing a verrucous lesion on the ventricular surface of the valve leaflet, round cell infiltration, endothelial proliferation, and stromal edema (x 60). (B) High-power magnification of a portion of the valve leaflet shown above (x 400) (J.A.M.A. 196:349, 1966).

Acute valvular lesions consisted of endothelial proliferation, round cell infiltration, and edema of the valve (Figures 1 and 2). Although fibrous adhesions between the valves and the mural endocardium developed in some of the older animals, there were no consistent morphologic variations with age. No histologic evidence of valvulitis was found in any of the control animals.

Coxsackievirus B_4 antigen was identified in the valves and mural endocardium, as well as in the myocardium, by immunofluorescent technics[4] in the virus inoculated animals. Tissue from control animals displayed only weak non-specific fluorescence.

Group II: Valvulitis was found in 6 of the 7 monkeys inoculated with group B_4 coxsackievirus. The gross lesions consisted of (1) verrucous aortic valvulitis in 2 animals, (2) verrucous mitral valvulitis in 3 animals (Figure 3), and (3) cicatricial thickening of the mitral valve leaflets and chordae tendineae with commissural adhesions (stenosis) and 2 animals (Figure 4).

Histologically, the valve tissue from the animals inoculated with group B_4 coxsackievirus displayed stromal edema, round cell infiltration, fibrocytic infiltration, increased basophilia and swelling of endothelial cells (Figures 5, 6, and 7).

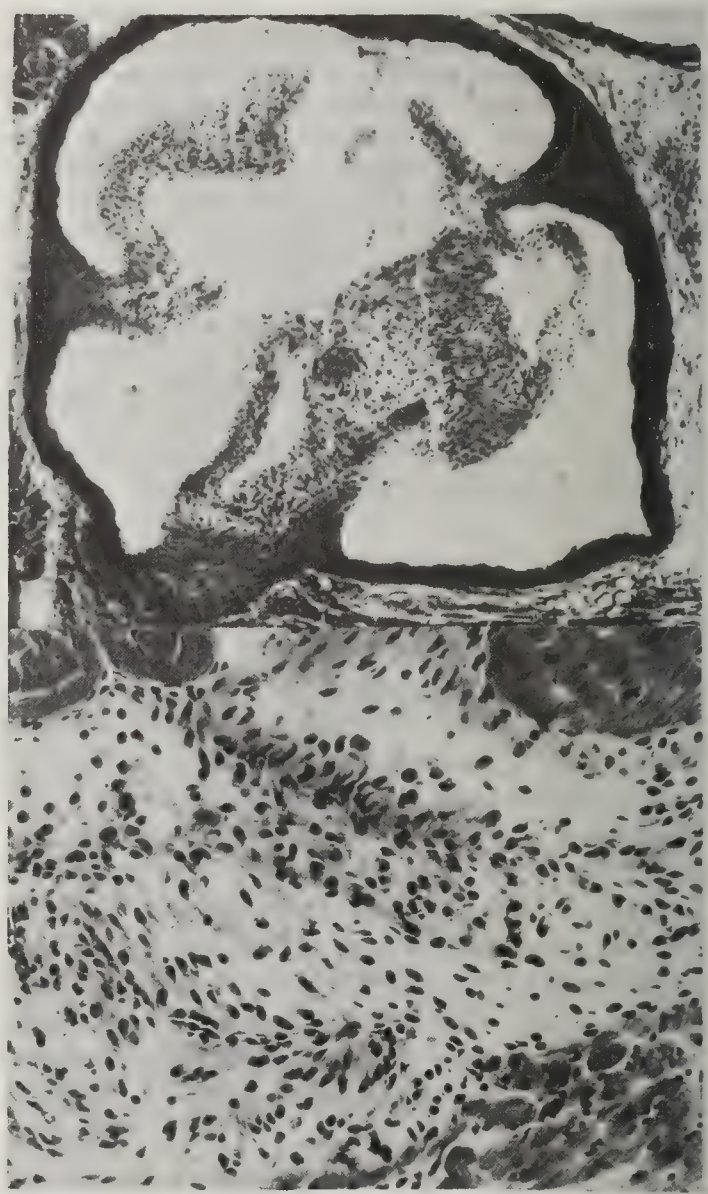


FIGURE 2.—Top: Aortic valve of a 20-day-old mouse killed 6 days after inoculation with coxsackievirus B_4 showing markedly thickened aortic cusps, round cell infiltration, and stromal edema (x 60). Bottom: Mural endocardium below the septal leaflet of the tricuspid valve of the same animal showing round cell infiltration of endocardial tissue (x 400) (J.A.M.A. 196:349, 1966).

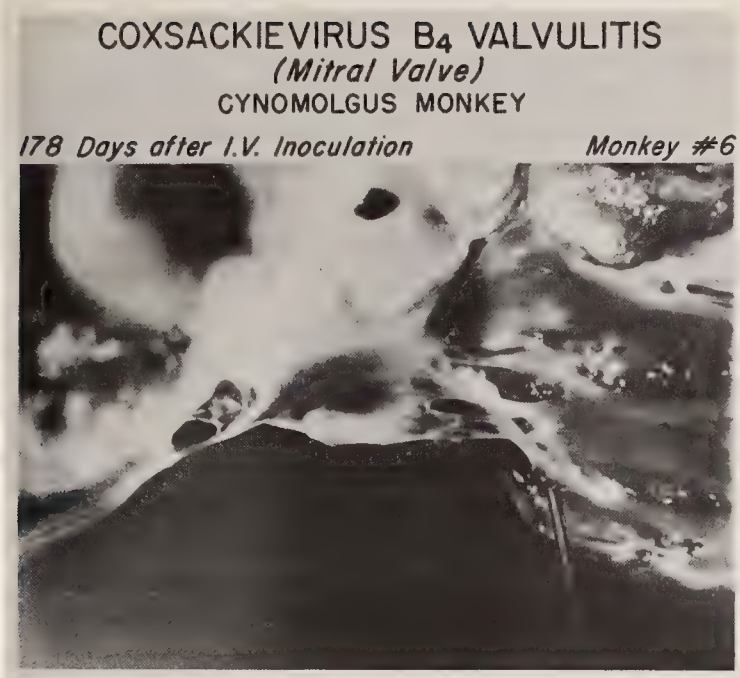


FIGURE 3.—Photograph showing verrucous lesion of the mitral valve associated with thickening of the valve in a cynomolgus monkey. The animal was killed 178 days after inoculation with coxsackievirus B_4 (Am. Heart J. 71:678, 1966).

Direct fluorescent antibody staining was carried out in 3 of the virus inoculated animals and in 1 control animal. Viral antigen was identified in valve tissue from the 3 virus-inoculated animals whereas valve tissue from the control animal was negative for viral antigen.

DISCUSSION

Physicians are often willing to attribute noncongenital chronic valvular disease to rheumatic fever even in the absence of a history of rheumatic carditis. Although occult or subclinical rheumatic fever does occur, it is unreasonable to assume that all instances of chronic valvular disease would occur secondarily to such unestablished entities.

Although clinicians and pathologists have not recognized acute and chronic viral valvulitis, valvular lesions have been produced in experimental animals by a variety of viruses. Pearce demonstrated that inoculation of rabbits with Virus III resulted in valvular lesions associated with infiltration of valve tissue with mononuclear cells containing characteristic inclusion bodies.[5] Pearce also found that vaccinia, pseudorabies and inflammatory fibroma viruses produced valvular lesions in acacia-prepared animals.[6,7] Kilham, Mason, and Davies produced endocarditis in African mongooses using the Mengo strain of encephalomyocarditis virus.[8] Lou, Wenner, and Kamitsuka found mural endocarditis in 4 and mitral valvulitis in 2 of 9 cynomolgus monkeys inoculated with group B_4 coxsackievirus.[9]

The studies described in this paper demonetrate that group B_4 coxsackieviral infection is often associated with acute valvulitis and mural endocarditis in mice. Furthermore, chronic experiments in cynomolgus monkeys demonstrated that viral infection may result in gross and microscopic valvular lesions which are strikingly similar to those of rheumatic valvulitis including fibrotic thickening of the valve leaflets, commissural adhesions, and shortening and thickening of the papillary muscles. In addition, immunofluorescent B_4 coxsackievirus antigen was found in valves as well as in the



FIGURE 4.—Photograph showing fibrosis and thickening of the mitral valve with commissural adhesions (stenosis) and thickening and shortening of the chordae tendineae in a cynomolgus monkey. The animal was killed 200 days after inoculation with coxsackievirus B_4 (Am. Heart J. 71:678, 1966).

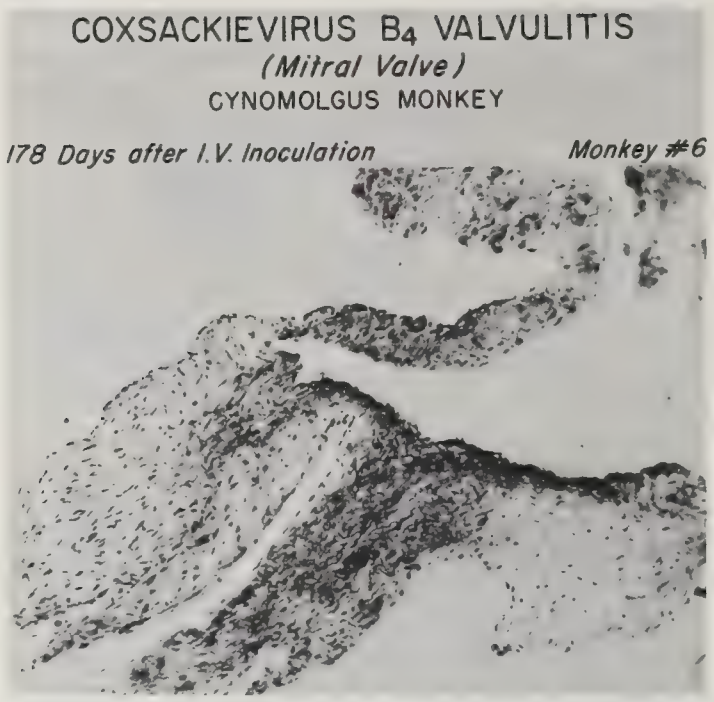


FIGURE 5.—Photomicrograph of mitral valve lesion shown in Figure 3, displaying round cell infiltration, fibroblastic proliferation and stromal edema (Am. Heart J. 71:678, 1966).

myocardium of virus-inoculated animals. Additional studies in this laboratory have demonstrated that Anitschkow's myocytes and Aschoff-like bodies may be identified in damaged tissue from cynomolgus monkeys inoculated with coxsackievirus B_4[10].

The experimental animal has proven to be an excellent model for testing the hypothesis that viral disease may be associated with acute and chronic valvular disease. It remains to be demonstrated that viral disease produces valvulitis in man. However, it is of interest that group B coxsackievirus antigens were identified by immunofluorescent technics in the myocardium of 17 and in the mitral valve of 3 of 55 patients at routine autopsy.[11]

There is a need for further study of the role of viruses in the pathogenesis of valvular disease in the human population. We can no longer accept the diagnosis of rheumatic valvulitis in the absence of a history of rheumatic fever without considering other etiologic possibilities.

SUMMARY

Although viruses are well known to produce pericarditis and myocarditis, they have not been recognized as a cause of acute and chronic valvulitis.

To test the hypothesis that viruses may produce endocarditis, over 200 mice and 7 cynomolgus monkeys were inoculated with coxsackievirus B_4. Mural endocarditis was found in 50% and valvulitis in 55% of the virus-inoculated mice. Valvulitis was found in 6 of the 7 virus-inoculated monkeys. In some instances the valcular lesions were strikingly similar to those of chronic rheumatic valvulitis in man.

It is suggested that viruses may play a role in the pathogenesis of human valvular disease and that it is no longer reasonable to assume that chronic valvular disease in the absence of an antecedent history of rheumatic carditis is always due to occult or subclinical rheumatic fever.

REFERENCES

1. BURCH, G. E., DEPASQUALE, N. P., SUN, S. C., MOGBGAB, W. J., and HALE, A. R. Endocarditis in

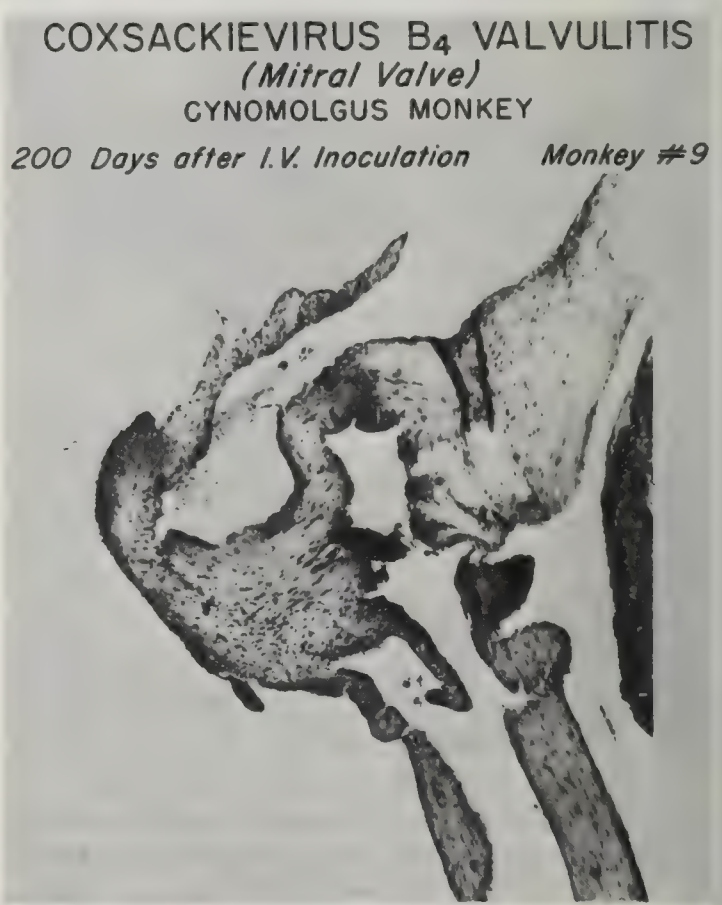


FIGURE 6.—Photomicrograph of the mitral valve lesion shown in Figure 4, displaying round cell infiltration, stromal edema, and thickening of the chordae tendineae (Am. Heart J. 71:678, 1966).

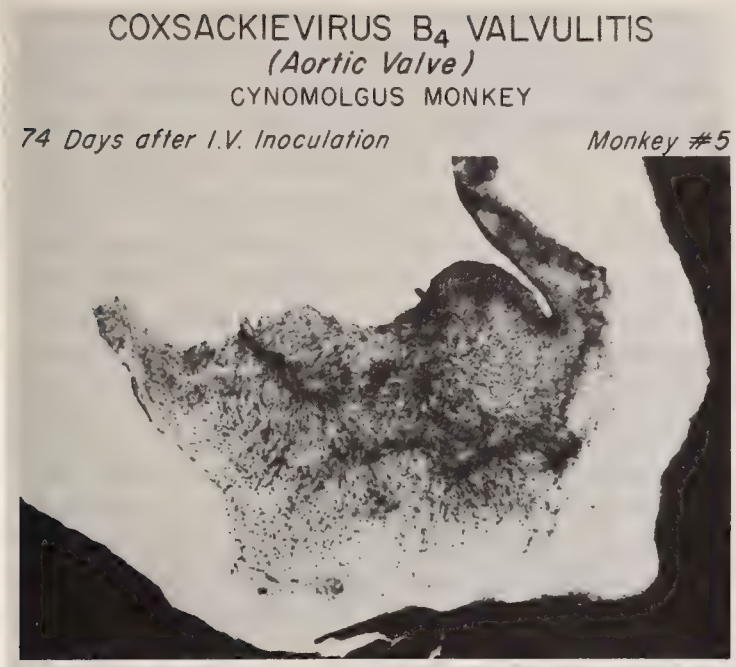


FIGURE 7.—Photomicrograph of a verrucous lesion of the aortic valve of a cynomolgus monkey inoculated with coxsackievirus B_4 74 days before autopsy (Am. Heart J. 71:678, 1966).

mice infected with coxsackie virus B_4. Science 151:447, 1966.

2. DEPASQUALE, N. P., BURCH, G. E., SUN, S. C., HALE, A. R., and MOBABGAB, W. J. Experimental coxsackie virus B_4 valvulitis in cynomolgus monkeys. Am. Heart J. 71:678, 1966.
3. Kibrick, S., and BENIRSCHKE, K. Acute aseptic myocarditis and meningoencephalitis in the newborn child infected with coxsackie virus group B, type 3. New England J. Med. 255:883, 1956.

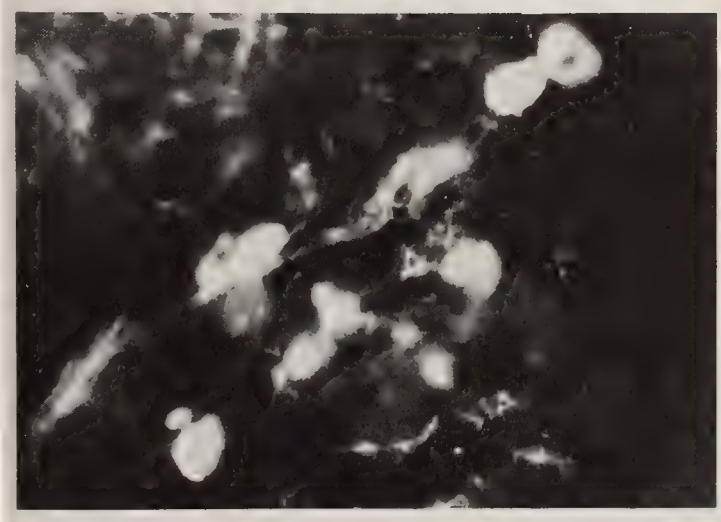


FIGURE 8.—Localization of coxsackievirus B_4 antigen in the mitral valve by direct immunofluorescence (x 320) (Am. Heart J. 71:678, 1966).

4. CHERRY, W. B., et al. *Fluorescent Antibody Technics in The Diagnosis of Communicable Diseases.* Publication 729, U. S. Public Health Service, 1960.
5. PEARCE, J. M. Cardiac lesions in rabbits produced by a filterable virus (Virus III). Arch. Path. 28:827, 1939.
6. PEARCE, J. M. Susceptibility of the heart of the rabbit to specific infection in viral diseases. Arch. Path. 34:319, 1942.
7. PEARCE, J. M. Heart disease and filterable viruses. Circulation 21:448, 1960.
8. KILHAM, L., MASON, P., and DAVIES, J. N. P. Host-virus relation in encephalomyocarditis (EMC) virus infections. II. Myocarditis in mongooses. Am. J. Trop. Med. 5:655, 1956.
9. LOU, T., WENNER, H. A., and KAMITSUKA, P. S. Experimental infections with coxsackieviruses. II. Myocarditis in cynomolgus monkeys infected with B_4 virus. Archiv. forsch. Virusforschung 10:451, 1960.
10. SUN, S. C., SOHAL, R. S., BURCH, G. E., CHU, K. C., and COLCOLOUGH, H. L. Coxsackie virus B_4 pancarditis in cynomolgus monkeys resembling rheumatic heart lesions. Brit. J. Exper. Path. 48:655, 1967.
11. BURCH, G. E., SUN, S. C., COLCOLOUGH, H. L., SOHAL, R. S., and DEPASQUALE, N. P. Coxsackie B viral myocarditis and valvulitis identified in routine autopsy specimens by immunofluorescent techniques. Am. Heart J. 74:13, 1967.

DISCUSSION

MORRIS POLLARD, University of Notre Dame: It seems that you have been able to detect antigen in these tissues for long periods of time after the initial inoculation. Either there is a virus there which has persisted, in perhaps an integrated form, or else your fluorescein type antiserum is not too specific. The solution to this would be, first of all, to isolate and recover the virus from these tissues, which would be the definitive step. A second aspect of this very interesting and, I think, important presentation is the clarification of the age of the inoculated animals because we do know that there is an age susceptibility to coxsackie infection which relates to the lethal effect of the virus.

DR. DEPASQUALE: Yes. I didn't mention it, but we had numerous control animals in both groups. In the monkey group, we had five controls, and in the mouse group, 100 controls. We've infected, over the years, over a thousand mice, and we have not found any positive immunofluorescence in the mice or in the monkeys.

There has always been some non-specific background fluorescence. We have isolated the virus from the hearts of mice up to ten days after initial inoculation. The virus cannot be isolated after ten days.

As far as the age of the monkeys, I don't have any idea because that's one of the problems of working with animals obtained from a biological supply house. We did maintain them in the vivarium for some six weeks to two months for observation, but we had no exact idea of their age. The mice were young, but they were not suckling mice. Daldorf established in order to produce the infection and some of the myopathy that occurs in the skeletal muscle, suckling mice are necessary, but we found that it really didn't make any difference whether these mice were newborn or two or three weeks old. We could still get myocarditis, although we couldn't get as much of the skeletal or the peripheral myopathy.

DR. POLLARD: There is another potential virus that has been associated with myocarditis and that is psittacosis. There is a question about whether or not it is a virus. Have you any additional information on that as a possible etiological agent?

DR. DEPASQUALE: Yes, someone mentioned that we might try it, but we had a lot of nerve doing this study, because none of us were virologists, and we didn't really know how to handle these animals. We had the advice of a virologist, but none of us wanted to experiment with psittacosis at the time. But it's true that it is another agent which might be examined. I think my plea really is: Let's not just assume it's all rheumatic fever and due to streptococcus. I think we should look for other things since we have this large number of patients without histories of rheumatic fever. Why should we accept it, just because our teachers told us (and their teachers told them) that it is due to rheumatic fever? Why shouldn't we look for other things?

CHAIRMAN PIERCE: At this time, since we are running ahead of schedule, I'd like to include Dr. G. D. Hsiung. She is from Yale University. She is in laboratory medicine. She is Chief of the Virology Laboratory at the V.A. Hospital in New Haven, Connecticut. Her interests are pathogenesis and epidemiology of viral diseases in laboratory animals. Viral latency is probably her primary interest. Her presentation will be titled "Virus Infections of 'Normal' Healthy Animals."

RESPIRATORY DISEASES OF DOGS, CALVES, AND SHEEP

R. A. Griesemer* and R. L. Farrell**

Naturally occurring diseases of the respiratory system are frequently encountered in experimental dogs, calves, and sheep. In a survey of 1,239 consecutively autopsied dogs, nearly all had anthracosis and the majority of adults had pneumoconiosis. Up to 90% of dogs in the Ohio and Missouri river valleys have histoplasmosis characterized by focal granulomatous pneumonia and hilar granulomatous lymphadenitis. The majority of dogs in the southern states have dirofilariasis that is not uncommonly associated with thromboembolism and arteritis of the pulmonary arteries. Young dogs that are immunosuppressed or severely stressed may develop lesions of infectious diseases during the experiment including the interstitial proliferative pneumonia of canine distemper, the focal fibrinonecrotic lesions of toxoplasmosis, and the tracheitis and bronchitis associated with herpesvirus, adenovirus, or parainfluenza virus.

The most frequently encountered respiratory disease in calves is pneumonia. The primary etiologic agent is considered to be a virus. Since cattle have a deficiency of lung fibrinolysins compared with other species, fibrinous pneumonias are not readily resolved and tend to persist with ultimate repair by fibrosis. It should be noted that in the calf and sheep, eructation provides an opportunity for inhalation of noxious gases from the rumen.

Nearly every sheep is parasitized. The larvae of *Oestrus ovis* cause chronic rhinitis and sinusitis and account for the exudate in the external nares of most sheep. Many sheep have pulmonary nematodiasis. *Dictyocaulus filaria* causes bronchiolitis and peripheral emphysema while *Mullerius capillaris* lives in the alveoli and causes lobular pneumonia. An occasional adult sheep develops pulmonary adenomatosis or caseous lymphadenitis.

INTRODUCTION

Naturally occurring diseases of the respiratory system are encountered in all animal species. While the quarantine and conditioning process prevents severely diseased animals from being used in experiments, clinically inapparent lesions may exist and interfere with the conduct of the experiment and with the interpretation of results.

The purpose of this report is to describe some frequently occurring, spontaneous, subclinical diseases of the respiratory systems, of dogs, calves and sheep—animals commonly selected for respiratory experiments. It is hoped that the following descriptions of the lesions will aid in their recognition and emphasize the limitations of these species for research purposes. The major disease problems that can be recognized clinically by the laboratory animal veterinarian are described in textbooks[1-3] and will not be discussed here.

RESPIRATORY DISEASES OF DOGS

Anthracosis

Since dogs live longer than most animal species employed in research and often share man's environment, it is not surprising that nearly every conventionally acquired dog has anthracosis. In a postmortem study of the lungs of 1,239 dogs, we found a direct correlation between the measured amount of pulmonary anthracosis and the age of the animals. As might be expected, there was also a direct correlation between amount of anthracosis and smoke pollution in the environment in which the dog lived as measured by dirt fall collection. While carbon pneumonoconiosis appears inocuous, its effects on clearance of other substances is unknown at this time. We have observed increased localization of histoplasma organisms and distemper inclusion bodies in anthracotic nodules.

Pulmonary Fibrosis

Pulmonary fibrosis also becomes increasingly common with advancing age. Patchy areas of

* National Center for Primate Biology, University of California Davis, California.

** Department of Veterinary Pathology, Ohio State University Columbus, Ohio.



FIGURE 1.—Postmortem radiograph of an old dog's lungs. The dense areas (arrow) are irregular foci of fibrosis. The tiny white discrete flecks are foci of mineralization that are representative of the density and distribution of healed lesions of histoplasmosis.

pleural and subjacent parenchymal fibrosis can be found in dogs over 6 to 8 years of age (Figures 1 and 2).

Pneumoconiosis

Dust pneumoconiosis in dogs has received little attention although the lesions are found regularly in dogs housed outdoors. Dust macules were reported in the lungs of experimental dogs by Goyal and Gupta.[4] The macules contained mainly silicates which were common air pollutants originating from a nearby desert area.

In central Ohio, a form of pneumoconiosis caused by dust from clay soils occurs in dogs, especially in the larger working breeds that live in a rural environment. The lesions are dust macules containing illite, a silica-containing compound common to clay soils in Ohio. The gray macules range in size from 1 mm in diameter to microscopic clusters of a few dust-laden macrophages. Thin, needlelike, birefringent spicules in the macules (Figure 3) are composed primarily of illite.

Histoplasmosis

Histoplasmosis in dogs[5] is endemic in the same regions of the United States as in man. The respiratory tract is the principal portal of entry for the disease in the dog as well as in man. In many instances, the pulmonary disease is subclinical. Healed or healing pulmonary lesions are found at necropsy as multifocal firm nodules from one to ten millimeters in diameter (Figure 4). The granulomatous foci, elicited by the fungus *Histoplasma capsulatum,* are surrounded and replaced by fibrous connective tissue. Often the centers of the lesions are caseous and partially mineralized (Figure 5). (It should be noted that tuberculous lesions in dogs, rarely, if ever, calcify). The final nodular lesion can be detected clinically only by radiography (Figure 1). The hilar bronchial lymph nodes

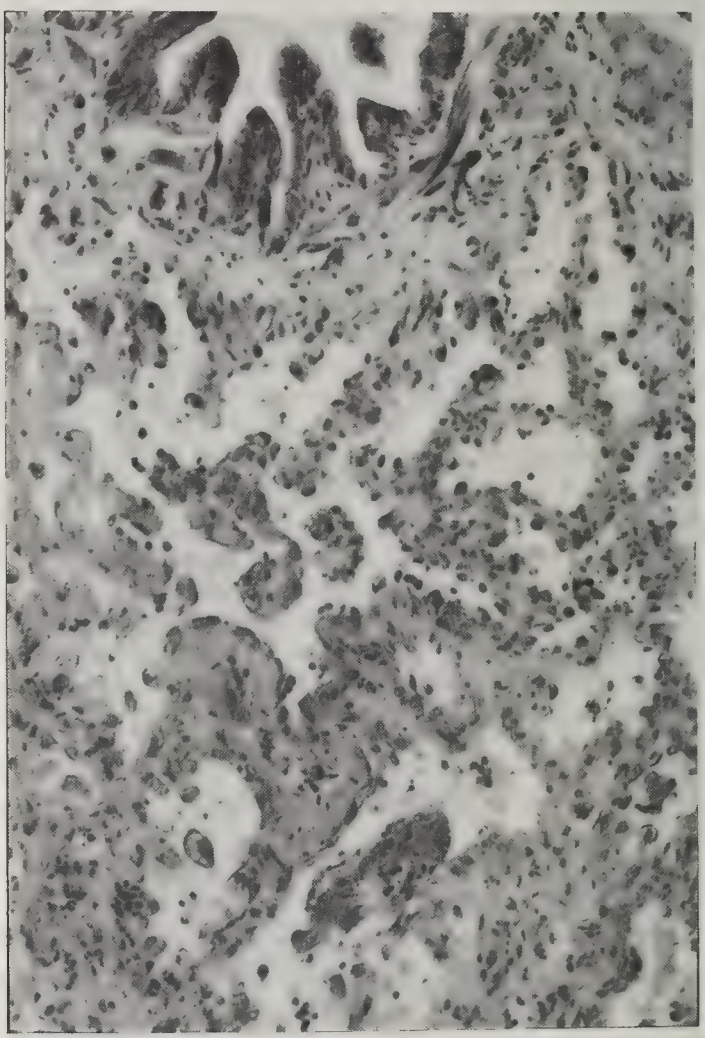


FIGURE 2.—Photomicrograph of the lung from the same dog as Figure 1 illustrating interstitial fibrosis. Masson's trichrome stain x 155.

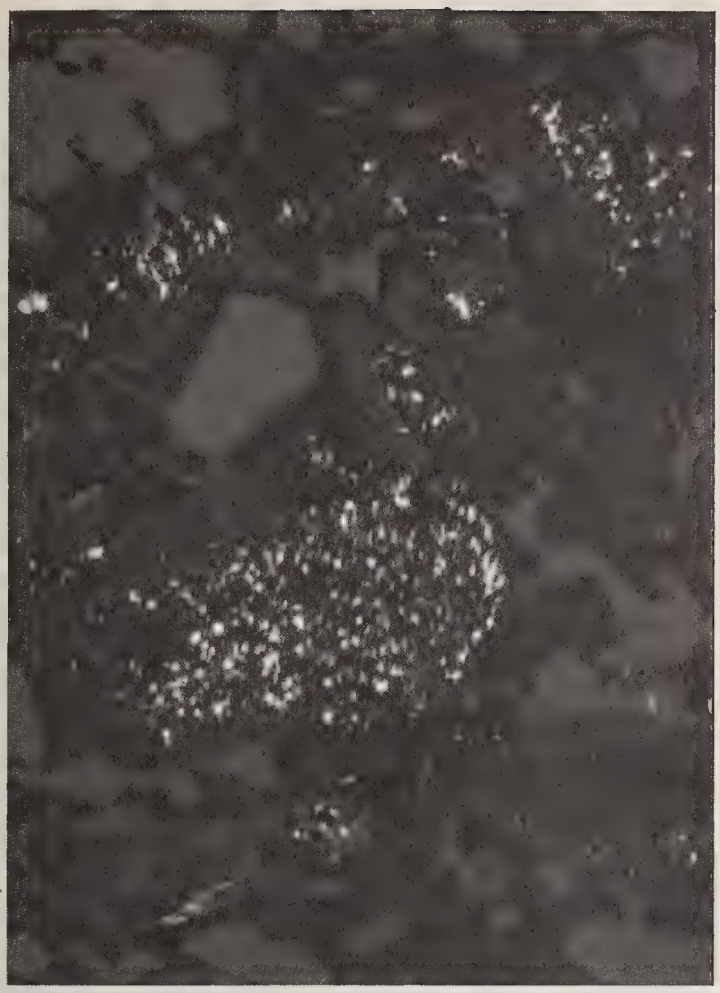


FIGURE 3.—Peribronchiolar dust pneumonoconiosis in a dog housed outdoors. The crystals of illite appear white in polarized light x 125.

may be enlarged and affected with the same type of lesion. Granulomatous hilar masses may compress the main bronchus or bronchi producing a chronic, dry, non-productive cough in an otherwise clinically healthy dog.

Dirofilariasis

Personal communications with personnel in laboratory animal facilities indicate that the majority of dogs in the southern states and many of those in the eastern coastal states are infected with *Dirofilaria immitis,* the dog heartworm. Although there is a low prevalence in northern and western states, the increasing mobility of the dog population makes it mandatory that all random-source dogs be screened for evidence of infection.

Present knowledge of the disease is reviewed by Pacheco.[6] Subclinical infections are associated with chronic pulmonary arteritis (Figure 6) characterized by villous intimal proliferation, medial hypertrophy, and arteriosclerosis.[7,8] Frequently pulmonary endarteritis and obstructive fibrosis develops around live adult worms. The severity of the disease is related in part to the number of parasites, but much variation occurs so that extensive pulmonary arteritis may occur with minimal numbers of circulating microfilariae and without congestive heart failure. Infrequently massive microfilariasis of the lung is associated with tiny granulomas, presumably an inflammatory response to dead microfilariae.

Other Infectious Diseases

Young dogs or dogs that are immunosuppressed or severely stressed may develop lesions of infectious diseases during the experiment. They include the interalveolar proliferative pneumonia of canine distemper,[9] the focal fibrinonecrosis of toxoplasmosis,[10] and the tracheitis and bronchitis associated with herpesvirus, adenovirus, or parainfluenza virus.[11] Space limitations prevent discussion of visceral larva migrans *(Toxocara canis)*, blastomycosis, coccidioidomycosis, tuberculosis, paragonamiasis, verminous tracheitis *(Oslerus osleri)*, and lymphoma of the lung.



FIGURE 4.—Multiple granulomas caused by *Histoplasma capsulatum* in the lung of a dog that had a chronic cough.

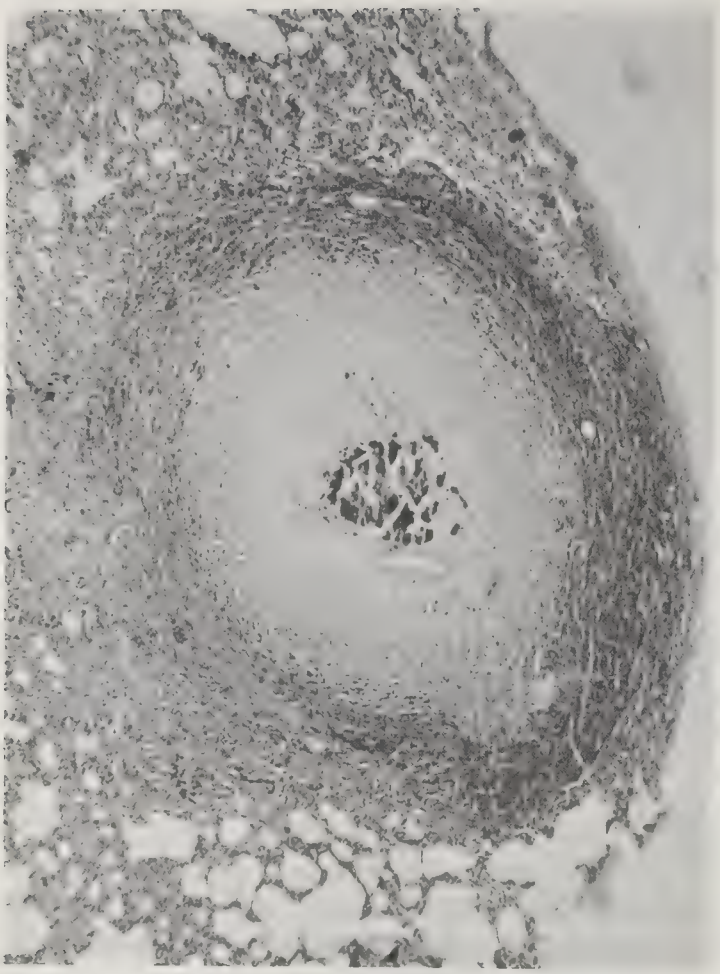


FIGURE 5.—Portion of a nearly healed granuloma of canine histoplasmosis with central caseation and mineralization. HE x 32.

RESPIRATORY DISEASES OF CALVES

Calf Pneumonia

The most important subclinical disease of calves is pneumonia.[12] Data on the incidence of calf pneumonia are lacking, although the impression gained from observations in slaughter houses and autopsy laboratories is that subclinical, chronic pneumonia occurs in the majority of calves from one week to six months of age.

The lesions occur in the dependent portions of the apical, cardiac, intermediate, and anterior diaphragmatic lobes, affecting from a few lobules up to one-fourth of the lung (Figure 7). The affected areas are dark red and sharply demarcated from the adjacent normal-appearing lung. Microscopically the lesions are characterized by peribronchial and perivascular accumulations of lymphoid cells and atelectasis (Figure 8) with varying admixtures of purulent broncho-pneumonia.

The causes of calf pneumonia and whether it represents more than one disease are still uncertain. The lesions are thought to be caused by a virus and secondary bacteria. Parainfluenza or adenoviruses are the most frequently incriminated viruses although they are infrequently isolated from the lesions of chronically diseased calves. *Pasteurella multocida* is most often isolated and *Cornyebacterium pyogenes* is not uncommon. The role of chlamydial organisms and mycoplasma as etiologic agents is still uncertain.

It is not clear whether bacterial pneumonias occur independently in calves or whether they represent complications of viral pneumonia. Occasionally severe suppurative bronchopneumonia occurs with a paracardial distribution in the dependent lobes similar to that of viral pneumonia. The lesions in calves with subclinical disease are somtimes accompanied by evi-

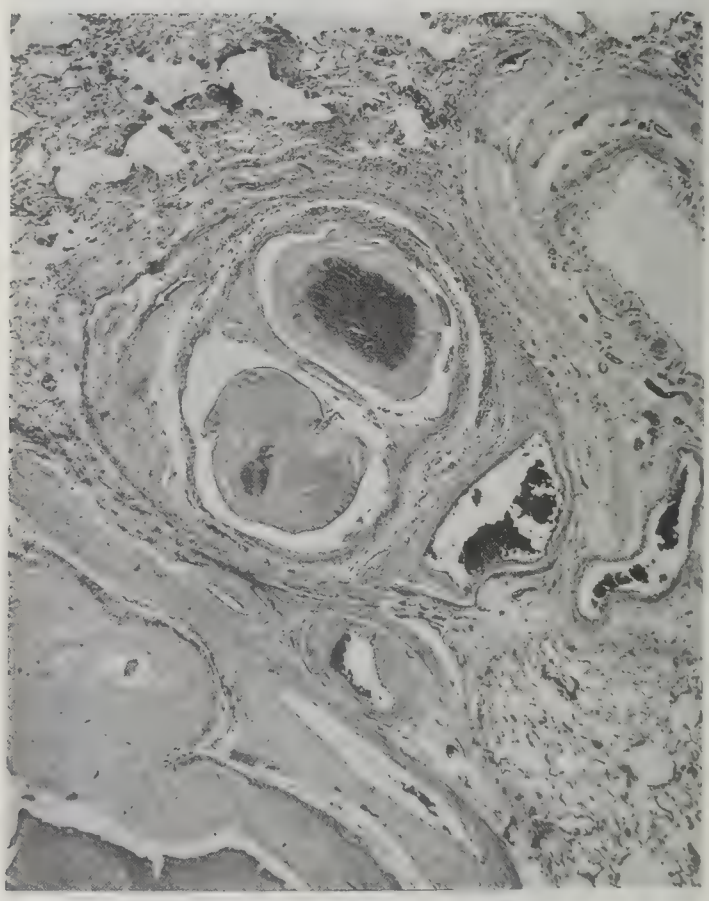


FIGURE 6.—Canine dirofilariasis. Branches of the pulmonary artery containing dead nematodes exhibit intimal hyperplasia and medial hypertrophy. HE x 32.



FIGURE 7.—Pneumonic consolidation (dark area) of nearly the entire apical lobe of the lung of a calf.

dence of chronicity including adhesive pleuritis, abscessation, bronchiectasis, and organization.

Fibrinous pneumonia presents a special problem in the bovine. Pneumonic exudates from all causes in calves nearly always contain fibrin (Figure 9). In contrast with other species, bovine lung tissue contains little fibrinolytic activity combined with large quantities of a protease inhibitor,[13,14] which result in delayed resolution of the fibrin deposited after tissue injury. The usual outcome of fibrinous pneumonia in the bovine is organization and ultimate repair by fibrosis. Fibrinous exudate could conceivably decrease the alveolar surface area exposed to noxious agents and thereby result in differences in the response of the bovine lung to injury. Another species variation of possible importance is that in the calf and sheep, eructation provides an opportunity for repeated inhalation of rumenal gases.

Calf Diptheria

Calf diptheria is the common name for necrotizing, pseudomembranous, ulcerative inflammation in the upper respiratory tract, caused by *Fusiformis necrophorus*. Lesions occur singly or multiply in the larynx, trachea, pharynx, or mouth. Typically, the lesions are well-demarcated with central raised, roughened, adherent necrotic exudate (Figure 10) which sometimes sloughs leaving a circular ulcer that heals by fibrosis. Overt clinical signs, when present, are persistent cough and occasionally dyspnea. While lesions of calf diptheria are no longer

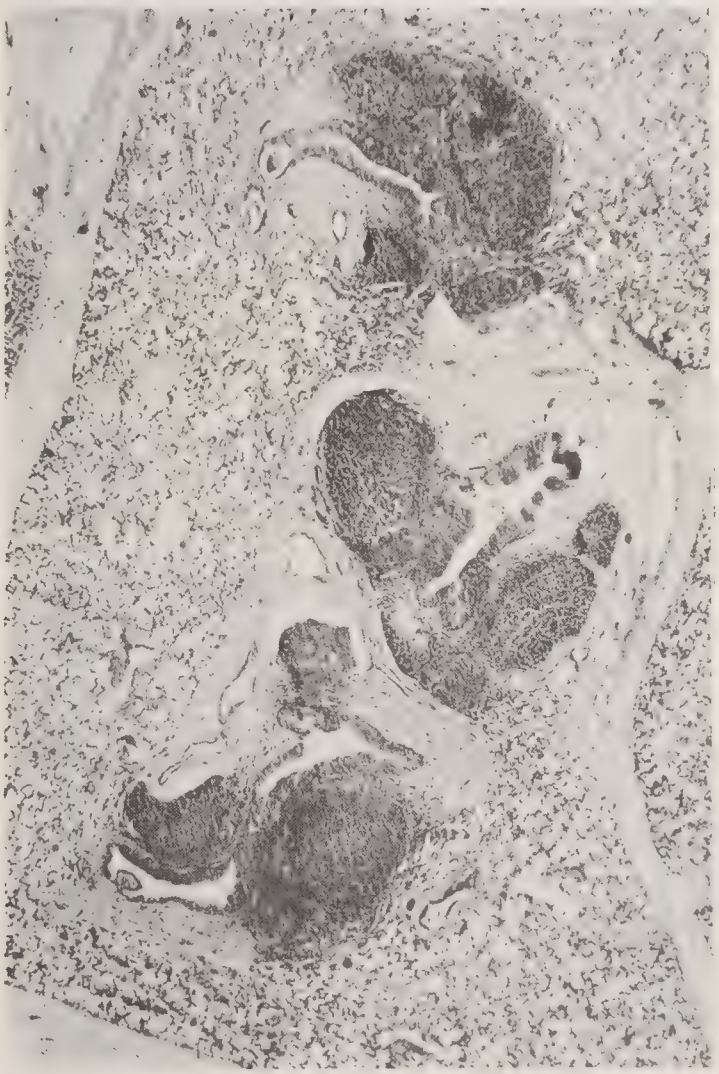


FIGURE 8.—Photomicrograph of pneumonic calf lung illustrating peribronchial accumulation of lymphoid cells.

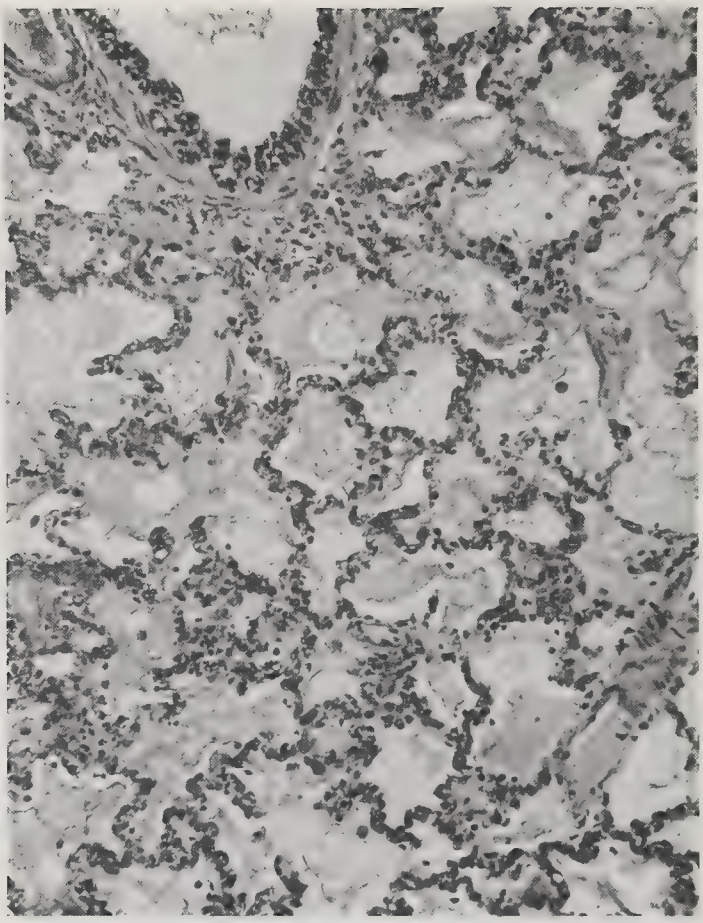


FIGURE 9.—Fibrinous pneumonia in a calf lung is related to a species difference in fibrinolytic activity. HE x 125.

common (about 0.2% of feedlot cattle) their effects on respiratory function are so great that investigators need be aware of the possible existence of the disease.

Emphysema

Emphysema in calves,[15] unlike that in man, is primarily interstitial. Severe interstitial emphysema is sometimes found as an artifact in older calves that have been euthanatized. The lesions are presumed due to agonal gasping and forceful respirations that over-distend the alveoli. The artifact can be avoided by careful euthansia.

Pulmonary Nematodiasis

Incidental infections by the bovine lungworm *(Dictyocaulus viviparous)* are recognized at surgery or autopsy when pink subpleural lobules in the diaphragmatic lobes fail to collapse. Further examination may reveal wedge-shaped, dark red lobules at the posterior margins of the diaphragmatic lobes (where in the bovine terminal bronchioles are near the pleural surface) and similar lobules scattered patchily through the other lobes about the major bronchi. Dissection of the lesions reveals long, slender, white worms in the bronchi along with frothy, mucinous exudate (Figure 11). Microscopically, the lesions are characterized by chronic catarrhal bronchitis and emphysema (pink lobules) or atelectasis and consolidation (red lobules).

RESPIRATORY DISEASES OF SHEEP

Rhinitis and Sinusitis

Chronic, serous and catarrhal rhinitis and sinusitis are characteristic of virtually all sheep. A major cause of rhinitis and sinusitis is myiasis, infestation with larval stages of *Oes-*

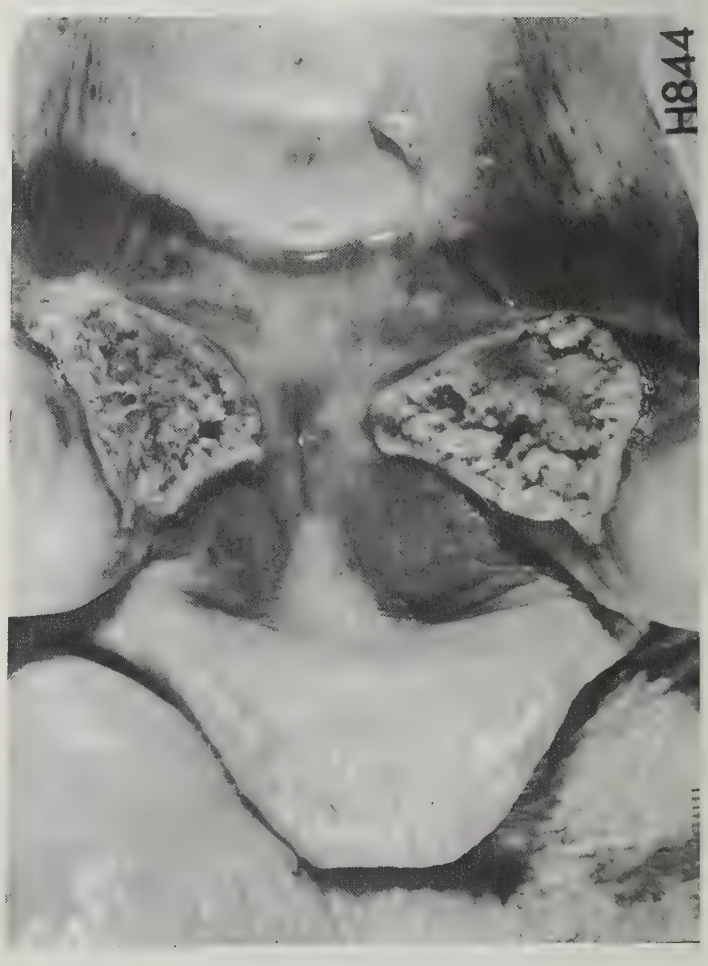


FIGURE 10.—Bilateral fibrononecrotic laryngitis in a calf caused by *Fusiformis necrophorus.*

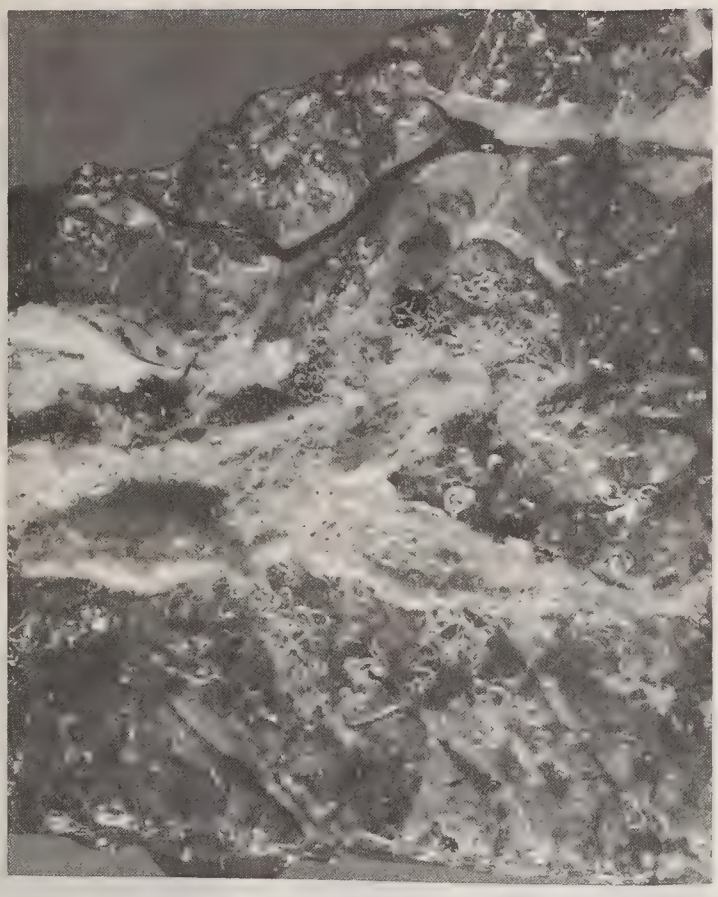


FIGURE 11.—Pulmonary nematodiosis in a calf. The bronchi were nearly occluded with masses of long, slender parasites *(Dictyocaulus viviparous)*.

trus ovis. Fly larvae gain entrance directly through the external nares as well as through dehorning wounds, periodontitis, and fractures. The larvae attach by their mouth parts to the mucosa and cause irritation resulting in excessive mucus secretion and exudative inflammation (Figure 12). The infestation is persistent and presumably uncomfortable but not of great consequence except when it interferes with the investigator's experiment.

Pulmonary Nematodiasis

Compared with calves, pneumonia is less frequent and parasitism more frequent in sheep. Pulmonary lesions caused by nematodes are common and widely distributed in random-source sheep. *Dictyocaulus filaria* causes bronchitis comparable to that caused by *D. viviparous* in cattle. The most ubiquitous of the lungworms, however, is *Mullerius capillaris*. In contrast with *Dictyocaulus* sp., *Mullerius* sp., is found in the alveoli where the adult parasites, larvae and ova provoke an enveloping granulomatous reaction (Figure 13) visible grossly as nodules (Figure 14). Older lesions frequently mineralize.

Caseous Lymphadenitis

Caseous lymphadenitis, suppurative inflammation of lymph nodes caused by *Corynebacterium ovis*, occurs as an inapparent infection in about 0.1% of sheep. The organisms gain entrance through shearing, castration, or docking wounds and produce isolated abscesses in lymph nodes (prescapular, mediastinal, and bronchial) and less often in visceral organs including the lung. The abscesses slowly enlarge to 10 to 15 cm and when sectioned, the inspissated exudate has a characteristic concentrically lamellated appearance (Figure 15).

Progressive Pneumonia and Adenomatosis

Progressive pneumonia is a transmissible and infectious disease (probably viral) of range sheep. Signs of wasting, occasional cough, and dyspnea on exertion develop slowly over months to years and affected sheep live 3 to 18 months after the onset of signs. The principal lesion is diffuse interstitial and proliferative pneumonia. Other sheep develop adenomatosis which rarely metastasizes to the bronchial lymph nodes. It is still unknown whether adenomatosis is an advanced form of progressive pneumonia or a separate entity. Progressive pneumonia accounts

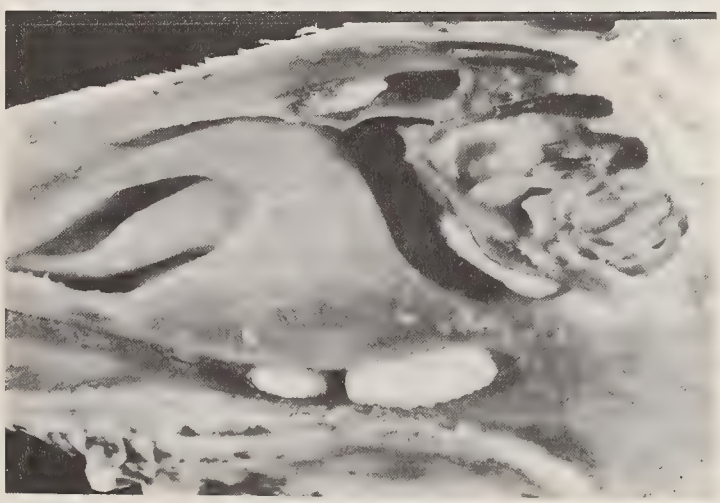


FIGURE 12.—Longitudinal section through the head of a sheep exposing larvae of *Oestrus ovis* in the nasal cavity and sinuses.

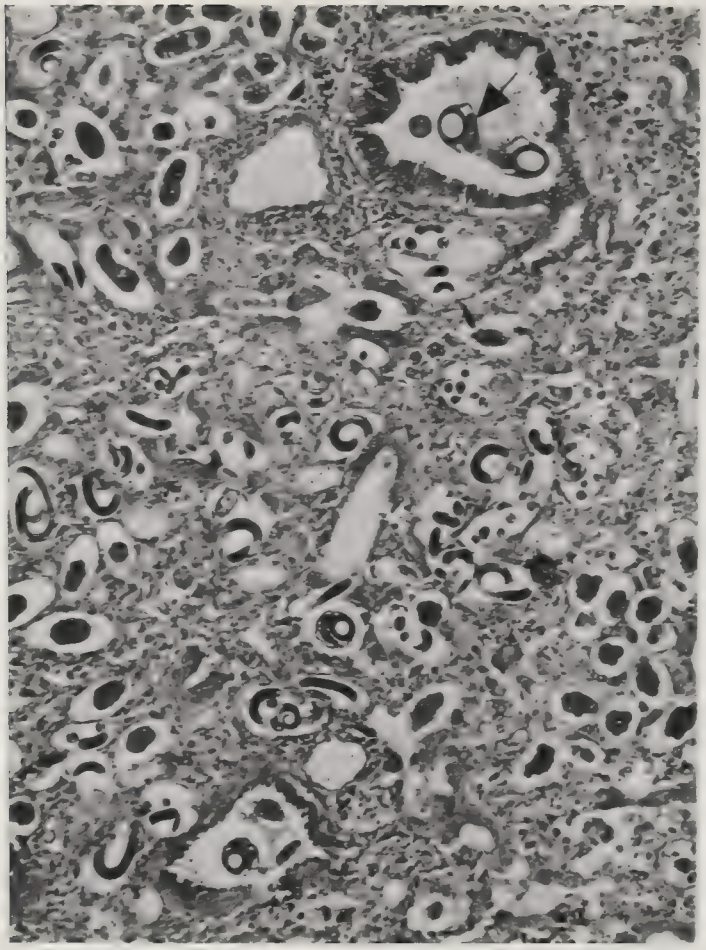


FIGURE 13.—Intra-alveolar verminous pneumonia in a sheep. Photomicrograph of a portion of a granulomatous nodule caused by adults, larvae, and ova of *Mullerius capillaris*. The arrow indicates a section of an adult in a bronchiole. HE x 125.

for about 3% of deaths of adult sheep although the prevalence may be as high as 10% in individual flocks.

SUMMARY

Each species of experimental animal has its own complement of spontaneous diseases. The majority of the respiratory diseases described, however, are infectious diseases that could be prevented or avoided through the use of techniques already established for germfree-derivation and subsequent maintenance under specific pathogen-free conditions.

REFERENCES

1. CATCOTT, E. J., Ed. *Canine medicine*. Amer. Vet. Publ., Inc., Wheaton, Ill., 1968.

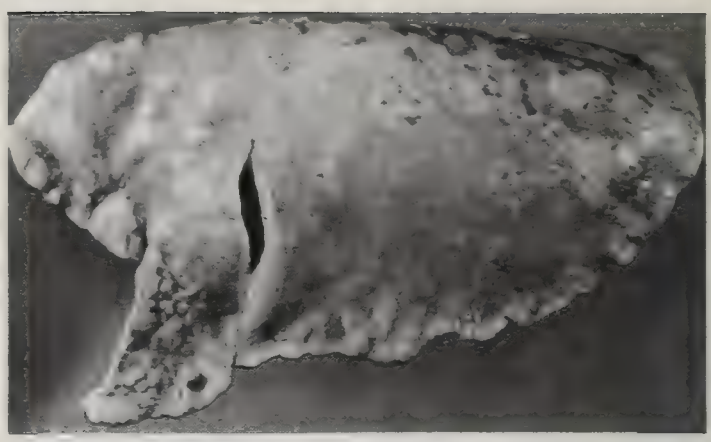


FIGURE 14.—Lateral surface of the ovine lung with nodular verminous pneumonia from which the photomicrograph in Figure 13 was taken.

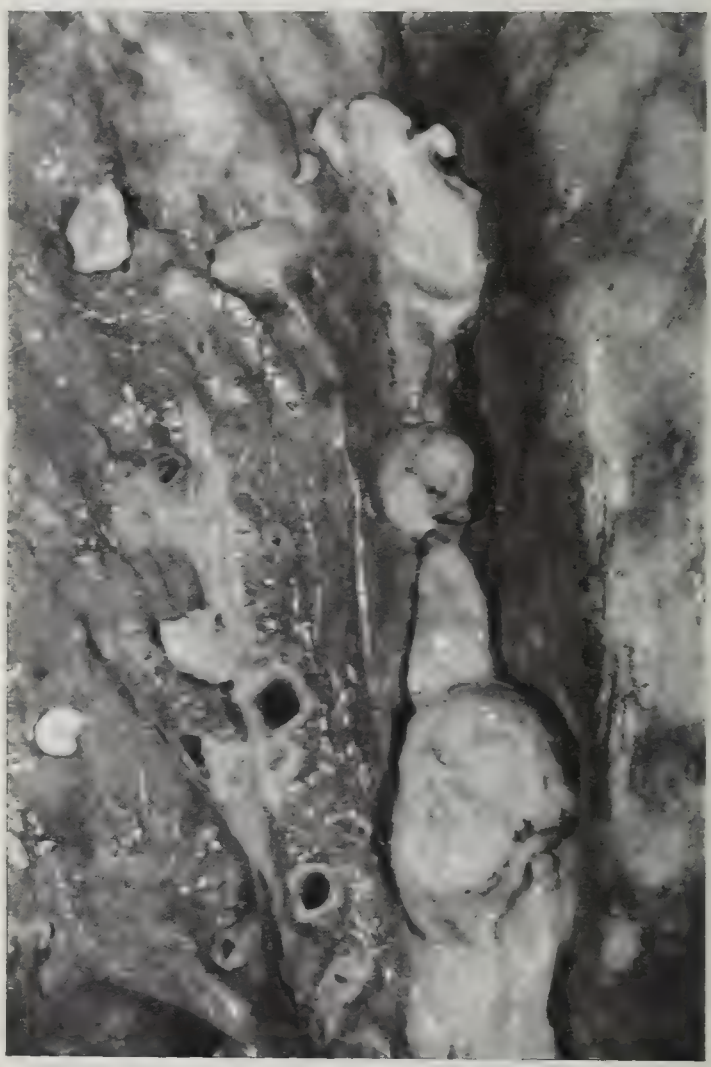


FIGURE 15.—Nodules of caseous necrosis in the lung and mediastinal lymph nodes of a sheep. The lamellated appearance of the lesions is characteristic of caseous lymphadenitis caused by *Corynebacterium ovis*.

2. Gibbons, W. J., et al., Eds. *Bovine medicine and surgery.* Amer. Vet. Publ., Inc., Wheaton, Ill., 1970.
3. Marsh, H. *Newsom's sheep diseases.* Williams and Wilkins, Co., Balt., 3rd Ed., 1965.
4. Goyal, R. K., and Gupta, D. P. Benign pneumoconiosis in monkeys in India. J. Indian Med. Assoc. 32:8–10, 1959.
5. Cole, C. R., Farrell, R. L., Chamberlain, D. M., Prior, J. A., and Saslaw, S. Histoplasmosis in animals. J. Amer. Vet. Med. Assoc. 122:471–473, 1953.
6. Pacheco, G. *Dirofilaria immitus:* A review of present knowledge. Proc. Natl. Conf. Res. Animals Med., NIH, 1972.
7. Adcock, J. L. Pulmonary arterial lesions in canine dirofilariasis. Amer. J. Vet. Res. 22:655–662, 1961.
8. Porter, W. B. Chronic cor pulmonale in dogs with "*Dirofilaria immitus*" (heart worms) infestation. Trans. Assoc. Amer. Phys. 64:328–334, 1951.
9. Jubb, K. V. F., and Kennedy, P. C. *Pathology of domestic animals.* Academic Press, New York, 2nd Ed., Vol. 1, pp. 215–221, 1970.
10. Capen, C. C. and Cole, C. R. Pulmonary lesions in dogs with experimental and naturally occurring toxoplasmosis. Path. Vet. 3:40–63, 1966.
11. Appel, M., et al. *Current status of canine respiratory disease.* Gains Vet. Symp., Gaines Dog Res. Center, White Plains, N.Y., 1970, pp. 15–23.
12. Jubb, K. V. F. and Kennedy, P. C. Pathology of domestic animals. Academic Press, New York, 2nd Ed., Vol. 1, pp. 221–225, 1970.
13. Astrup, T., Glas, P., and Kok, P. Lung fibrinolytic activity and bovine high mountain disease. Proc. Soc. Exp. Biol. Med. 127:373–377, 1968.
14. Astrup, T., Glas, P., and Kok, P. Thromboplastic and fibrinolytic activity in lungs of some mammals. Lab Invest. 22:381–386, 1970.
15. Epling, G. P., Kainer, R. A., and Card, C. S. *Bovine pulmonary emphysema.* Proc. Natl. Conf. Res. Animals Med., NIH, 1972.

VIRUS INFECTIONS OF "NORMAL" HEALTHY ANIMALS

G. D. Hsiung and N. S. Swack*

Longitudinal survey studies of virus infections in normal healthy animals, including primates and non-primates, showed that both groups may harbor latent viruses. The greatest number and variety of viruses were recovered from tissues of rhesus and green monkeys. A herpesvirus was consistently isolated from inbred strain 2 guinea pigs but only occasionally from the Hartley strain. Rabbits, hamsters and cattle were commonly free of virus infections in their kidney tissues. Two of ten lots of horse kidney cultures examined showed a herpesvirus infection. Circulation of simian viruses in monkeys during captivity was observed to have occurred. Green monkeys acquire SV40 and foamy virus type 1 from rhesus; whereas the latter acquire cytomegalovirus from the green monkeys. Both monkey species may acquire SV5 infection from man.

INTRODUCTION

Previous studies by a number of investigators have demonstrated the presence of a variety of latent viruses in simian tissues.[1–9] The recognition of these viral agents in primate tissues has proven to be important especially when the tissue cells are used for the preparation of virus vaccines, or when the animals are used for medical research. A number of the viruses isolated from "normal" monkeys were shown to be oncogenic. These included: (1) SV40, a papovavirus, isolated from apparently healthy rhesus monkeys,[10] (2) several types of simian adenoviruses isolated from healthy African green monkeys, as well as from macaca monkeys,[11] and (3) herpesviruses isolated from normal squirrel and owl monkeys.[12,13]

During the past several years, we have conducted survey studies on the prevalence of virus infections in "normal" healthy animals, including monkeys, horses, cattle, rabbits, guinea pigs and hamsters. The comprehensive results of these studies are included in this discussion.

* Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut, and Veterans Administration Hospital, West Haven, Connecticut.

Virus Infections in Primates and Non-Primates

Primary cell cultures prepared from primate and non-primate kidney tissues were examined for the presence of latent viruses (Table I). Cultures in the primate group were prepared from the kidney tissues of rhesus and green monkeys; and in the non-primate group from the kidneys of guinea pigs, rabbits, hamsters, cattle and horses. It was noted that the primate cultures, especially rhesus monkeys, showed a higher percentage of viral agents than the non-primate group. Of the 124 rhesus monkeys, 65 viruses were isolated; and of the 108 green monkeys studied, 35 showed the presence of viral agents in their kidney tissues.

A herpes-like virus was repeatedly isolated from inbred strain 2 guinea pigs and on two occasions was also isolated from Hartley guinea pigs.[14] Since the virus persisted for the lifetime of the infected animals and transplacental transmission of the virus in guinea pigs was demonstrated, it is speculated that most likely all strain 2 guinea pigs harbor the virus.[15] No virus was isolated from the kidney cell cultures prepared from 211 New Zealand white rabbits,

TABLE I.—*Latent Virus Infections in Primary Cell Cultures Derived from "Normal" Healthy Animals*

August 1969—June 1971

Animals Studied	No. Studied	No. showed latent virus	% of virus infection
Primates			
Rhesus Monkeys	124	65	52
African Green Monkeys	108	35	32
Non-Primates			
Guinea Pigs—Strain 2 (inbred)	82	72	88
Hartley Strain	198	2	1
Rabbits—New Zealand White	211	0	0
Hamsters—Syrain Golden	16	0	0
Cattle	50	0	0
Horses	10*	2	20

* Nine horses were examined during January–May 1966.[16]

16 golden hamsters, and 50 cattle. A herpesvirus was obtained from the kidney cultures of two of the ten horses studied.[16] These results indicate that certain monkeys held in captivity harbor a greater number of viruses than most laboratory or domestic animals.

Comparison of Virus Types Recovered from Different Monkey Species

The virus types recovered from different monkey species varied from time to time and from laboratory to laboratory. Table II shows a comparison of the different types of viruses isolated from the two monkey species that are listed in Table I, together with the percentage of monkeys infected with each virus type. In both the rhesus and green monkeys, members of the foamy virus group were the most frequent isolates, being 31% and 20%, respectively. The incidence of measles and SV5 isolations was low in both groups. Rhesus tissues were the only source of SV40, and cytomegalovirus was found only in the green monkeys of the samples examined during the study period.[17,18]

Circulation of Simian Viruses in Monkeys During Captivity

In our earlier studies, when green and rhesus monkeys were quarantined for a period of time, a lower incidence of SV5 isolation from their kidneys was obtained than from monkeys that came directly from the field.[8] On the other hand, the green monkeys showed a higher percentage of SV40 infection the longer they were kept in captivity.[19,20]

The sources of virus infection in monkeys are often obscure. Some of these agents, such as, SV40, are undoubtedly viruses of rhesus monkeys, and green monkeys acquired SV40 infection only after contact with the former (Figure 1a). A similar situation has been noted with foamy virus type 1 infection. Of a total of 184 rhesus and 64 green monkeys examined, from January 1967 to February 1969, foamy virus type 1 was isolated in cell cultures prepared from 42 rhesus monkeys, but none was isolated from the green monkeys.[17] However, a 4% seroconversion to foamy virus type 1 was found in green monkeys which were housed in close proximity to the rhesus monkeys for 30 days or longer (Figure 1b). In contrast, studies on cytomegalovirus infections in captive monkeys showed that the green monkeys were highly susceptible to this virus, both by virus isolation and serologic tests. Of 108

TABLE II.—*Comparison of Virus Types Isolated from Kidney Cell Cultures of Green Monkeys and Rhesus Monkeys*

August 1969—June 1971

	Species of Monkey	
	Rhesus	Green
Total No. Studied	124	108
virus type (%)		
Foamy	31	20
Measles	2	1
SV5	2	1
SV40	17	0
Cytomegalovirus	0	8
Mixed Infection	1*	0

* Doubly infected with SV40 and Foamy virus type 1.

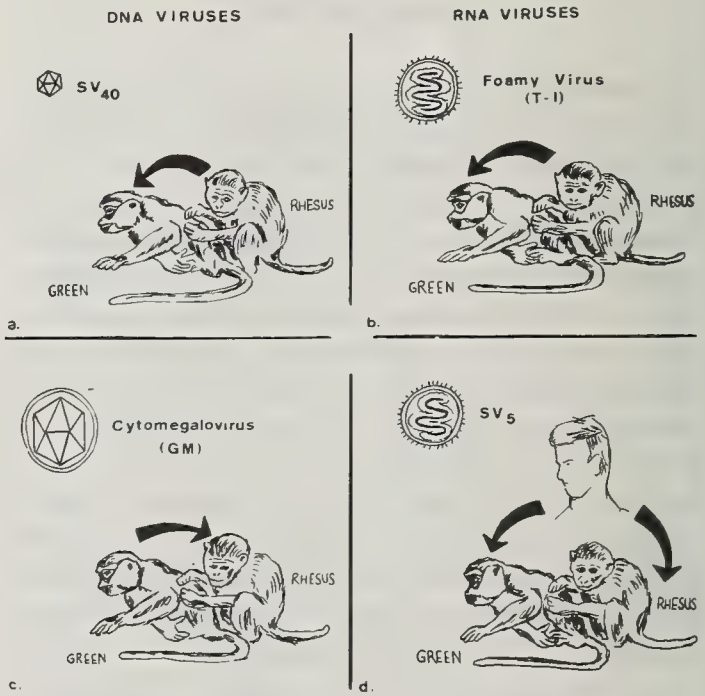


FIGURE 1.—During captivity, when different monkey species were housed together or close to each other, the following observations on the spread of virus infection have been made:

Green monkeys appear to acquire SV40 (a) and foamy virus type 1 (b) from infected rhesus monkeys, but transmit cytomegalovirus (c) to the latter; both monkey species appear to acquire SV5 infection from man (d).

green monkeys studied, all of them possessed high levels of neutralizing antibody to a strain of African green monkey cytomegalovirus; whereas only low levels of antibody titers were obtained in rhesus monkeys.[18] Seroconversion of the rhesus monkeys to green monkey cytomegalovirus occurred during captivity (Figure 1c). The origin of SV5 or parainfluenza 5 virus infection in monkeys is as yet unclear.[21] Monkey sera obtained at the site of capture did not contain SV5 antibody. In a study of 351 paired sera collected from 161 green and 190 rhesus monkeys during a 30–90 day quarantine, it was found that 45% of the rhesus and 56% of the green monkeys showed antibody conversion to SV5.[8] Similar results were obtained by other investigators using either paired or single serum samples.[22–24] It appears that monkeys may have acquired SV5 infection after contact with man in a manner similar to that reported for measles virus infection in monkeys[25] (Figure 1d).

SUMMARY

Longitudinal survey studies on virus infections in "normal" healthy animals showed that a greater number of virus isolations were made from simian tissues than from the non-primate tissues.

In the non-primate laboratory animals studied, rabbit kidney cells were commonly found free of virus infection but the strain 2 inbred guinea pigs consistently harbored a herpes-like virus.

In the domestic animal group, no virus was obtained from kidney cell cultures prepared from the 50 cattle examined but a herpesvirus was isolated from the kidney cultures of two of the ten horses examined.

The virus types isolated from monkeys varied from time to time and laboratory to laboratory. Circulation of simian viruses occurred during captivity. Green monkeys appeared to acquire SV40 and foamy virus type 1 from rhesus; whereas the latter apparently acquired cytomegalovirus from green monkeys. Both monkey species may contact SV5 from man.

REFERENCES

1. Rustigian, R., Johnston, P., and Reihart, H. Infection of monkey kidney tissue cultures with virus-like agents. Proc. Soc. Exp. Biol. Med. 88: 8–16, 1955.
2. Hull, R. N., Minner, J. R., and Smith, J. W. New viral agents recovered from tissue cultures of monkey cells. I. Origin and properties of cytopathic agents, SV1, SV2, SV4, SV5, SV6, SV11, SV12, and SV15. Am. J. Hyg. 63:204–215, 1956.
3. Malherbe, H., and Harwin, R. Seven viruses isolated from vervet monkey. Brit. J. Exp. Path. 38: 539–541, 1957.
4. Ruckle, G. Studies with the monkey-intra-nuclear-inclusion-agent(MINIA) and foamy agent derived from spontaneously degenerating monkey kidney cultures. I. Isolation and tissue culture behavior of the agents and identification of MINIA as closely related to measles virus. Arch. ges. Virusforschung 8:139–166, 1958.
5. Sweet, B. H., and Hilleman, M. R. The vacuolating virus SV40. Proc. Soc. Exp. Biol. Med. 105: 420–427, 1960.
6. Hsiung, G. D., and Gaylord, W. H. The vacuolating virus of monkeys. I. Isolation, growth characteristics and inclusion body formation. J. Exp. Med. 114:975–986, 1961.
7. Hsiung, G. D., and Atoynatan, T. Incidence of naturally acquired virus infections of captive monkeys. Am. J. Epidemiol. 83:38–47, 1966.
8. Hsiung, G. D. Latent virus infections in primate tissues with special reference to simian viruses. Bact. Rev. 32:185–205, 1968.
9. Hull, R. N. The simian viruses. Virology Monographs 2:2–66, 1968.
10. Eddy, B. E., Borman, G. S., Grubbs, G. E., and Young, R. D. Identification of the oncogenic substance in rhesus monkey kidney cell cultures as simian virus 40. Virology 17:65–75, 1962.
11. Hull, R. N., Johnson, I. S., Culbertson, C. G., Reimer, C. B., and Wright, H. F. Oncogenicity of the simian adenoviruses. Science 150:1044–1046, 1965.
12. Melendez, L. V., Hunt, R. D., Daniel, M. D., Blake, B. J., and Garcia, F. G. Acute lymphocytic leukemia in owl monkeys inoculated with *Herpesvirus saimiri*. Science 171:1161–1163, 1971.
13. Daniel, M. D., Melendez, L. V., King, N. W., Fraser, C. E. O., Barahona, H. H., Hunt, R. D., and Garcia, F. G. Herpesvirus aotus: A latent herpesvirus from owl monkeys *(aotus trivirgatus)*: Isolation and characterization. Proc. Soc. Exp. Biol. Med. 138:835–845, 1971.
14. Hsiung, G. D., Kaplow, L. S., and Booss, J. Herpesvirus infection of guinea pigs. I. Isolation, characterization and pathogenicity. Am. J. Epidemiol. 93: 298–307, 1971.

15. Lam, K. M., and Hsiung, G. D. Herpesvirus infection of guinea pigs. II. Transplacental transmission. Am. J. Epidemiol. 93:308–313, 1971.
16. Hsiung, G. D., Fischman, H. R., Fong, C. K. Y., and Green, R. H. Characterization of a cytomegalovirus isolated from spontaneously degenerated equine kidney cell culture. Proc. Soc. Exp. Biol. Med. 130:80–84, 1969.
17. Swack, N. S., Schoentag, R. A., and Hsiung, G. D. Foamy virus infection of rhesus and green monkeys in captivity. Am. J. Epidemiol. 92:79–83, 1970.
18. Swack, N. S., Liu, O. C., and Hsiung, G. D. Cytomegalovirus infections of monkeys and baboons. Am. J. Epidemiol. 94:397–402, 1971.
19. Meyer, H. M., Jr., Hopps, H. E., Rogers, N. G., Brooks, B. E., Bernheim, B. C., Jones, W. P., Nisalak, A., and Douglas, R. D. Studies on simian virus 40. J. Immunol. 88:796–806, 1962.
20. Stiles, G. E. Serologic screening of rhesus and grivet monkeys for SV40 and the foamy viruses. Proc. Soc. Exp. Biol. Med. 127:225–230, 1968.
21. Hsiung, G. D. Parainfluenza 5 virus. Infection in man and animals. Progr. Med. Virology 14:241–274, 1972.
22. Tribe, G. W. An investigation of the incidence, epidemiology and control of simian virus 5. Brit. J. Exp. Path. 47:472–479, 1966.
23. Kalter, S. S., Ratner, J., Kalter, G. V., Rodriguez, A. R., and Kim, C. S. A survey of primate sera for antibodies to viruses of human and simian origin. Am. J. Epidemiol. 86: 552–568, 1967.
24. Kalter, S. S., and Heberling, R. L. Comparative virology of primates. Bact. Rev. 35:310–364, 1971.
25. Meyer, H. M., Jr., Brooks, B. E., Douglas, R. D., and Rogers, N. G. Ecology of measles in monkeys. Am. J. Dis. Children 103:307–313, 1962.

ACKNOWLEDGMENT

This study was supported in part by the U. S. Public Health Service Research Grant AI-08648 and Research Contract NIH-69-2045 from the National Institute of Allergy & Infectious Diseases, National Institutes of Health, and Veterans Administration Research Funds.

SIGNIFICANCE OF SIMIAN VIRUSES

S. S. Kalter*

In the pursuit of developing better systems for the study of human disease, investigators have gradually worked their way up the phylogenetic scale to the current widespread use of monkeys and apes. Approximately 100,000 nonhuman primates are imported annually into the United States with an equal number, or more, used elsewhere in the world as laboratory animals. Their importation and usage has not been without problems. Some of these have been overt and others have been at a level not easily recognized nor detected except in retrospect or by means of special efforts. Most important is the realization that many individuals do not recognize or perhaps accept the fact that these animals are biologic entities and thus have a potential danger by virtue of the "normal" flora and fauna, especially viral. Precise information as to what constitutes the normal viral flora of simians is still not known. Most available data are derived from captive animals and only limited information is available on the various species as they exist in nature. Approximately 20–30 species of simians, broadly separated into Old and New World monkeys and apes, are now in use in the laboratory and these vary considerably in their viral content as well as their susceptibilities to viruses of animal origin. These animals have been responsible for introducing such virus diseases as herpes B *(Herpesvirus simiae)*, Marburg and hepatitis, into laboratory personnel. In the field, contact with simians has resulted in the transmission of such diseases as yellow fever and Kyasanur forest to humans. Of equal importance is the transmission of a number of simian viruses, at times with numerous fatalities, from one monkey species to another: Yaba and Yaba-like, simian hemorrhagic disease, a number of Herpesviruses, especially *H. saimiri*, and so on. Lastly is the exchange of human and simian viruses between all the different primate species that are only detected by serologic changes. The extent of pathogenesis associated with this last point is practically completely unknown. Unquestionably all of these effects have a certain influence and significance on the health of man and simians, experimental economics, as well as on the interpretation and outcome of the final experiment. This significance will be discussed.

*Division of Microbiology and Infectious Diseases Southwest Foundation for Research and Education San Antonio, Texas 78284.

INTRODUCTION**

In searching for animal models to be utilized for the study of human disease, an increasing number of investigators have turned to the nonhuman primate as an attempt to close the phylogenetic gap which exists when other animal systems are employed. However, in so doing one must be aware of the existence of certain problems and the possible relationship these problems may present in the final interpretation of results. One must also be concerned with the very pragmatic aspect of the health and well-being of the human and animal populations. It is to these considerations that we address ourselves at this time.

We will make no attempt to delineate or define those experimental needs requiring use of nonhuman primates, but it is worth mentioning that such usage should be scrutinized extremely carefully. Only too frequently exotic and rare (some almost to the point of extinction) animals are used for experimentation that could be well satisfied by use of another laboratory animal. A number of the nonhuman primates are now included among the endangered species and their use should be carefully considered!

MONKEYS AND APES

Table I lists various species of nonhuman primates that are used in the laboratory. Certain species, for example the rhesus monkey *(Macaca mulatta)*, are more commonly employed than others. In certain instances size, cost and availability, as in the case of the gorilla *(Gorilla gorilla)*, precludes extensive employment. In many instances the choice of simian employed for one or another investigative situa-

**This study has been partially funded by U.S.P.H.S. grants #RR05519, RR00278, RR00361, RR00451 and contracts #NIH 69–93 and WHO grant Z2/181/27.

TABLE I.—*Survey of Nonhuman Primates Being Maintained on 1 January 1971*

(Prepared by R. W. Thorington, Jr.)
ILAR News XV, No. 1, October, 1971, p. 7

Species [1]	No. of Labs.	No. of Animals
Pongidae		
Gorilla *(Gorilla gorilla)*	2	17
Chimpanzee *(Pan troglodytes)*	24	443
Orangutan *(Pongo pygmaeus)*	1	27
Hylobatidae		
Black gibbon *(Hylobates concolor)*	1	2
White-handed gibbon *(Hylobates lar)*	3	14
Gibbon *(Hylobates sp.)*	3	23
Siamang *(Symphalangue syndactylus)*	1	2
Cercopithecidae		
Gray-cheeked mangabey *(Cercocebus albigena)*	1	1
Black mangabey *(C. aterrimus)*	1	2
Agile mangabey *(C. galeritus)*	1	3
Sooty and white-collared mangabeys *(C. torquatus)*[2]	4	158
Green monkey *(Cercopithecus aethiops)*	31	2,057
Moustached monkey *(C. cephus)*	1	1
Blue and Sykes monkeys *(C. mitis)*[3]	4	8
Talapoin monkey *(C. talapoin)*	1	34
Patas monkey *(Erythrocebus patas)*	8	168
Stump-tailed macaque *(Macaca arctoides)*	53	809
Assamese macaque *(M. assamensis)*	1	1
Formosan macaque *(M. cyclopsis)*	2	103
Crab-eating macaque *(M. fas cicularis)*	45	2,689
Japanese macaque *(M. fuscata)*	4	120
Rhesus macaque *(M. mulatta)*	148	23,300
Pig-tailed macaque *(M. nemestrina)*	36	1,327
Celebes macaque *(M. nigra)*[4]	4	77
Bonnet macaque *(M. radiata)*	10	441
Barbary ape *(M. sylvanus)*	1	15
Hybrid macaques *(Macaca)*	3	6
Savannah baboon *(Papio cynocephalus)*[5]	47	1,535
Sacred baboon *(Papio hamadryas)*	2	9
Drill *(Papio leucophaeus)*	1	5
Mandrill *(Papio sphinx)*	1	4
Hanuman langur *(Presbytis entellus)*	1	45
Gelada baboon *(Theropithecus gelada)*	2	32
Cebidae		
Night monkey *(Aotus trivirgatus)*	28	1,061
Black-handed spider monkey *(Ateles geoffroyi)*	1	3
Black spider monkey *(Ateles paniscus)*	4	9
Spider monkey *(Ateles sp.)*	5	11
Red Uakari *(Cacajao rubicundus)*	1	1
Dusky titi *(Callicebus moloch)*	1	34
Royal titi *(Callicebus torquatus)*	1	4
White-fronted capuchin *(Cebus albifrons)*	26	255
White-throated capuchin *(Cebus capucinus)*	2	11
Tufted capuchin *(Cebus apella)*	9	177
Capuchin *(Cebus sp.)*	2	14
Wooly monkey *(Lagothrix lagotricha)*	6	22
Squirrel monkey *(Saimiri sciureus)*	86	3,941
Callitrichidae		
Goeldi's marmoset *(Callimico goeldii)*	2	13
Pygmy marmoset *(Cebuella pygmaea)*	3	44
Brown-headed tamarin *(Saguinus fuscicollis)*	1	317
Moustached tamarin *(S. mystax)*	5	462
Black and red tamarin *(S. nigricollis)*	8	1,359
Cotton-top tamarin *(S. oedipus)*	14	390
Lorisidae		
Thick-tailed galago *(Galago crassicaudatus)*	8	451
Senegal galago *(G. senegalensis)*	9	60
Slow loris *(Nycticebus coucang)*	7	47
Potto *(Perodicticus potto)*	1	10

TABLE I.—Continued

Species [1]	No. of Labs.	No. of Animals
Lemuridae		
Ring-tailed lemur *(Lemur catta)*		45
Brown lemur *(L. fulvus)*[6]	3	91
Black lemur *(L. macaco)*[6]	3	23
Mongoose lemur *(L. mongoz)*	3	24
Mouse lemur *(Microcebus murinus)*	2	9
Tupaiidae		
Tree shrews *(Tupaia sp.)*[7]	4	46

TABLE II.—*Viruses Recovered From Nonhuman Primates And Their Biological Characteristics*

Simian Adenoviruses
(DNA, ether resistant, 70–80 nm, acid stable, nuclear inclusions, HA±)
SV1, 11, 15, 17, 20, 23, 25, 30, 31, 32, 33, 34, 36, 37, 38—rhesus, cynomolgus
SA7, 17, 18—African green
V340, AA153—baboon
C–1, PAN 5, 6, 7—chimpanzee
Sq M–1—squirrel monkey

Simian herpesviruses
(DNA, ether sensitive, 120–250 nm, acid labile, nuclear inclusions, HA—)
Type A
H. simiae (B virus)—rhesus
SA8—African green
H. Tamarinus (platyrrhinae)—squirrel
SMV—spider
Type B
SA6—African green
Herpes saimiri (?)—squirrel
Liverpool vervet agent (?)—African green, patas

Simian myxoviruses
(RNA, ether sensitive, 150–250 nm, acid labile, cytoplasmic and nuclear inclusions, HA+)
SV5—rhesus (man?)
SV41—cynomolgus
SA10—African green

Simian Papovaviruses
(DNA, ether resistant, 40–57 nm, acid stable, nuclear inclusions, HA—)
SV40—rhesus
SA12 (?)—African green

Simian Picornaviruses
(RNA, ether resistant, 18–38 nm, acid stable, cytoplasmic inclusions, HA±, $MgCl_2$ stabilized)
Enteroviruses
SV2, 6, 16, 18, 19, 26, 35, 42, 43, 44, 45, 46, 47, 49—rhesus, cynomolgus
SA5—African green
A13—baboon
Unclassified
SV28—rhesus
SA4—African green

Simian poxviruses
(DNA, ether or chloroform sensitive, 200–325 nm, acid labile, cytoplasmic inclusions, HA±)
Vaccinia—Variola group
Monkey pox—cynomolgus
Moluscum contagiosium group (?)
Yaba
Yaba-like disease (benign epidermal pox, 1121)—rhesus

Simian Reoviruses
(RNA, ether resistant, 70–77 nm, acid stable, cytoplasmic inclusions, HA+, $MgCl_2$ stabilized)
SV12 (Reo 1), SV59 (Reo 2)—rhesus
SA3 (Reo 1)—African green

TABLE II.—Continued

Foamy Virus (presently included with the myxoviruses)
(RNA (?), ether sensitive, 100–300 nm, acid labile, no inclusions, HA—)
Type 1—rhesus, African green
Type 2—African green
Type 3—African green
Type 4—Squirrel
Type 5—galago
Type 6—chimpanzee
Type 7—chimpanzee
Miscellaneous viruses
SA11—African green
SHF—rhesus
Marburg agent—African green

tion is simply one of availability. Usefulness, in the sense of satisfying a known experimental need, is only too frequently secondary.

For practical purposes, nonhuman primates are subdivided into Old and New World monkeys and apes with the former being further subdivided into Asian and African animals. This separation, while based upon a geographic existence, has the practical effect of suggesting (with a number of exceptions) which simian viruses may be encountered. The SV series[1–3] were derived primarily from Asian macaques, whereas the SA numbered viruses[4] were recovered from African monkeys *(Cercopithecus aethiops)*. Additional viruses have been since recovered from numerous other species: chimpanzee, baboon and several of the South American monkeys. Current indications strongly suggest that, in addition to the geographic separation, other biologic parameters exist and should be investigated or considered. A phylogenetic relationship is recognized by differences in susceptibility to various infectious agents and immunologic reactivities. It is important to remember that monkeys and apes, along with man, are primates, and as such are biologic entities fully endowed with the various biologic complexities that are associated with animals at this phylogenetic level.

SIMIAN VIRUSES

Approximately 75 viruses have been recovered from tissues and body fluids of various nonhuman primates (Table II). It will be noted that these are, for the most part, counterparts of virus families that have been described previously as recovered from other animal tissues. Thus there are: adenoviruses, picornaviruses (although no simian rhinoviruses have been reported), herpesviruses, poxviruses, myxoviruses, papovaviruses, reoviruses and a number that are of undetermined classification. Whereas these viruses are biologically related to previously described human and other animal viruses, they are antigenically distinct. Established human and other animal viruses have been recovered frequently from various simians.

A number of the original reports and reviews are available describing the recovery and characteristics of these agents[5–10] and they will not be discussed at this time. Of the many viruses recovered from nonhuman primates, the vast majority are apparently of low pathogenicity for their original host. The effect on alien hosts has been examined only to a very limited extent. It is here, however, that apparent problems arise, but again the extent has not been defined. In certain instances, it is known that crossing of the species barrier by one or another of these agents does result in an increased pathogenicity, even to the extent of producing death. Fortunately the number of simian viruses in this category, as far as man is concerned, are few in number—*Herpesvirus simiae,* Marburg agent. Certain of the herpesviruses are also lethal for species of monkeys other than the original host—*H. tamarinus* (isolated from the squirrel monkey) is fatal for the owl monkey and marmoset; *H. hominis* may produce fatalities in various species of monkeys and apes. To this list should be added those viruses, especially in general, yellow fever and Kyasanur forest. These two viruses are highly pathogenic for man and monkey alike. Also, there is always the question of the unknown pathogenic potential simian viruses may have. For example, thousands of individuals have received live SV40 virus in conjunction with their poliovirus vaccine. This virus, when introduced into newborn hamsters, is carcinogenic. No known tumors have ever been recorded as related to this virus in its original simian host nor in any of these recipients. However, the question of possibility still remains unanswered.

Following is a brief review of pertinent aspects of these infections to acquaint those investigators utilizing nonhuman primates in

their research and who are not familiar with various epidemiologic factors associated with the transmission of these simian agents to man and other animals.

Human infection and deaths have been reported for:

H. simiae—this virus, more commonly known as B virus, produces in its original host, *Macaca* sp., a disease similar to that seen in man as a result of infection with herpes simplex *(H. hominis)*. Transmitted to man by a monkey bite, although other routes are suspected, a fatal disease results. The few recognized survivors have been reduced to the level of a vegetable existence. Antigenically *H. simiae* is closely related to *H. hominis* but little, if any, protection is offered by the presence of antibody to this virus even though serologic cross reactions are noted. A vaccine has been prepared by one commercial company but as yet it has not been licensed.

Marburg virus (green monkey disease)—an unclassified virus considered generally to be a rhabdovirus because of its size and bullet shape. An "epidemic" due to this virus occurred in Germany (Marburg-Frankfurt) and Yugoslavia in 1967 and thus far has not been reported again. Both countries had received African green monkeys from the same shipment out of Uganda. Animal handlers were not involved. Laboratory technicians handling monkey tissues and blood as well as hospital staff (nurses and pathologists) in contact with patient tissues and blood were those that became ill. Approximately 30 cases occurred with 8 deaths. Venereal transmission is known to have occurred in one instance. As indicated, this disease has not reappeared and there is still some question regarding its exact epidemiology. Conflict in interpreting serologic results that suggest an African simian reservoir has developed, with some investigators indicating that such a potential exists where as other groups have not been able to substantiate this finding.[11–18] Additional collaborative studies concerning this problem are underway in several laboratories.

In addition to these human infections, several of the simian viruses have produced widespread disease among nonhuman primate colonies. Among these that should be mentioned are:

Poxviruses—three separate and distinct poxviruses have been recovered from nonhuman primates (*Macaca* sp.). Of these, monkeypox is considered to be a natural disease of simians and has been reported as occurring in a variety of monkeys and apes[19–30] as well as in man.[31] This virus is very closely related to the variola-vaccinia complex and nonhuman primates may be protected by vaccination with vaccinia.[32–34] The epizootiology of this disease is still suspect and it is conceivable that monkeypox infection of simians results from contact with a poxvirus infection of another animal. This premise is based upon serologic data indicating a lack of antibody to poxviruses in simian populations. As mentioned above, cases of human disease as a result of infection with monkeypox virus occurring in unvaccinated humans in Africa have been recently reported.[35]

Yaba virus was first described by Bearcroft and Jamieson as an outbreak of subcutaneous tumors occurring in rhesus monkeys in Yaba, Nigeria.[36] Rhesus and cynomolgus monkeys are highly susceptible but African species are only susceptible when reared in countries outside Africa. This, along with the high susceptibility of Asian species, would suggest that Yaba virus is common to Africa with widespread infection and subsequent immunity developing.[37–39] Humans are susceptible to experimental or accidental infection with development of histiocytomas similar to those seen in monkeys. Regression of tumors occurs in 6–8 weeks. Since this virus is not antigenically related to vaccinia-variola, vaccination with one of these viruses is of little value.

Yaba-like virus infection of monkeys has been reported from several monkey colonies.[35] The original source of this virus, however, is unknown. Clinically this disease resembles a milder form of Yaba, running its course in about 2 weeks. The lesions are tumor-like in appearance, occurring on the skin. Human infection has been reported and, as in Yaba disease, vaccination with vaccinia virus is of no value.

Simian Hemorrhagic Fever virus—several epizootics with high mortality rates have occurred as a result of this virus in colonies of macaques in the U.S., England and Russia.

This disease is not to be confused with the many hemorrhagic diseases occurring in man and other animals due to a multitude of different agents. Mortality is extremely high in susceptible animals but the high degree of species selectivity is unexplainable at this time. Other species of monkeys and apes do not have antibody to SHF and this cannot, therefore, explain their resistance.

Herpesviruses (other than *H. simiae*)—simian herpesviruses distinct from *H. simiae* are now recognized and these, in species other than the original host, produce a diversity of clinical diseases. It is now well established that herpesviruses in their natural hosts usually produce inapparent or latent infections rather than overt disease. Thus, *H. tamarinus*,[40–45] a herpesvirus of squirrel monkeys referred to at times as marmoset herpes virus, or *H. platyrrhinae*, is highly lethal for the owl monkey and marmoset. Studies with other simian species will undoubtedly demonstrate a wider spectrum of susceptibilities to this virus. The spider monkey virus (SMV) of Lennette[46] produced characteristic lesions in its original host: herpetic lesions on lips, nose, gums, tongue, palate and brain. With death resulting, this appears to be contrary to usual events for a natural host. In explanation, two possibilities come to mind: 1) the spider monkey may not be the original host although the evidence (increase in number of seropositive spider monkeys with age) suggests this, and 2) as in man, herpesviruses do occasionally produce fatal disease in their original host, although, admittedly this is rare.

The studies of Melendez and his coworkers[47–52] pertaining to simian herpesviruses of New World monkeys may indeed prove to be a major contribution. These investigators have now isolated a number of herpesviruses from such South American monkeys as the squirrel *(H. saimiri)*, spider *(H. ateles)*, marmoset *(H. saguinus)*, owl *(H. aotus)* and possibly others. Several of these viruses have been shown to produce a lymphoproliferative disease in species of monkeys other than the original host. These findings are the first record of primate cancer-producing viruses and extend the series of oncogenic viruses closer to man. Of additional importance is the recognition that the carrier state may develop in these animals similar to that known to occur in man.[53] Thus, *H. tamarinus* has been found to produce carriers in the marmoset in spite of the occurrence of a high mortality as a result of herpesvirus infections.

Two other herpesviruses reported as producing disease have been recovered from African monkeys: Liverpool vervet monkey virus[54] and patas monkey virus.[55] Probably these viruses are the same but more studies are needed. The LVV produces a severe and distinctive disease in vervet monkeys and it would appear that this species is not the natural host for this agent. The patas agent produces clinical and pathologic responses in this monkey similar to that described for the vervet agent: skin rash on face, trunk, proximal areas of the limbs. Rash begins as macules and changes to papules and then to vesicles. Death occurs within 48 hours after appearance of the rash.

Foamy viruses—this virus group is mentioned at this time because of their nuisance value rather than known pathogenicity. Their presence in simian tissues, at times 100% of the kidney cell preparations contain these viruses, makes them unsuitable for vaccine preparation or experimental work. Appropriate classification of the agents in this group is not clear and many investigators consider them along with the myxoviruses or as paramyxoviruses or even pseudomyxoviruses. Many of their biologic characteristics are similar to the myxoviruses but not to the extent of hemagglutinating erythrocytes. The seven current prototypes have been recovered from kidneys of many different species of monkeys and apes (see Table 2). Their presence appears to be more of economic concern than of disease production.

Myxoviruses—under the simian myxoviruses two that are currently included therein, i.e. SV5 and SV41, have not been discussed. SV5 is probably not a simian virus although admittedly it is very frequently recovered and is a nuisance for those involved in monkey tissue culture work. SV41 is found so infrequently that it is not considered to be of major consequence. This virus does produce some CNS pathology and death in monkeys after experi-

mental inoculation but there is no evidence that SV41 causes disease under natural conditions.

NONSIMIAN VIRUSES IN SIMIANS

Following are a number of nonsimian viruses capable of infecting simians:

Arboviruses—Several arboviruses are known to produce infection and/or death in monkeys and apes: yellow fever, Kyasanur forest and dengue. Of questionable concern is the fact that monkeys and apes all come from areas known to be endemic arbovirus areas. It is surprising that there has not been a greater recognized involvement of these animals with arboviruses. All these viruses are vector borne and antibody surveys[56–58] certainly indicate that infection of nonhuman primates (and probably disease) results from many arboviruses. Yet actual knowledge regarding the infectivity of arboviruses for simians has been limited.

Yellow fever virus is highly lethal for man and monkey alike. Jungle yellow fever is dependent upon involvement of monkeys in the infectious cycle. Here the disease is undoubtedly enzootic in monkeys (probably other forest animals as well) and is transmitted and maintained from one animal to another by forest dwelling mosquitoes. Different species of monkeys are involved in the various endemic areas. In man and monkeys, yellow fever infection is usually inapparent. Epidemics occur with irregular frequency and may result in high attack and mortality rates in both the human and simian populations. It is conceivable, although Public Health regulations are designed to prevent this, that infected animals might be imported. Vaccination or appropriate mosquito-proof housing is effective but frequently both mechanisms may break down. The available vaccines are effective but the chick embryo preparation is highly labile and prevention of its deterioration is essential to maintain its efficacy.[59]

Work et al.[60] isolated a virus from a dead langur monkey found in the Kyasanur forest in South India. Infection of both man and monkeys with this agent results from a tick *(Haemaphysalis)* bite. Shah[61] has shown the presence of virus in experimentally lactating monkeys and infection has been transmitted to a baby monkey by breast feeding. Antibody surveys have determined that survivors among the different monkey species do occur. Because of lack of vectors, this disease probably would not spread in the U.S. but again it is possible to import infected animals. However, this disease is similar to other tick-borne virus encephalitides and the epidemiology of this group is in need of clarification.

The status of the numerous other arboviruses is still not clear. Serologic studies suggest that many viruses very probably cause infection and/or disease in primates. The extent to which this happens is still unknown. It is fairly certain that disease outbreaks are infrequent or more would have been detected. It is also possible that lack of interest and study has contributed to the limited knowledge concerning infection and/or deaths of monkeys and apes in nature. In this regard it is interesting to note that a true simian arbovirus has yet to be described.

Hepatitis—In 1961, Hillis[62] reported an outbreak of hepatitis in Air Force personnel handling chimpanzees. This finding of a chimpanzee-human relationship has now been well documented with numerous reports demonstrating the occurrence of hepatitis in individuals closely associated with this species—usually young, newly imported animals. The epidemiology of this disease is not clear and it is possible that the chimpanzee becomes infected as a consequence of associating with humans (or their products) in their natural habitat. Experimental infection of other species of simians, for example patas monkeys[63–67] and marmosets,[68–71] has also been reported but needs further study.

The recent description of an agent (Australia antigen) by Blumberg et al.[72] that appears to be closely related to if not the same as this virus has also precipitated studies in monkeys and apes. Serologic investigations of sera from limited numbers of nonhuman primates suggest with fair certainty that the apes (chimpanzees, orangutans, and gibbons) have circulating antigen and perhaps antibody. The status of monkeys is not as clear, but in general they do not appear to have antibody. Deinhardt[69] has re-

cently reviewed the status of nonhuman primates and Australia antigen.

Myxoviruses—the myxoviruses that are significant, as far as simians are concerned, appear to be of human origin.

Measles (Rubeola) infections are rather common among different species of nonhuman primates and the virus may be encountered in various tissues of these animals. For example, Ruckle [73] recovered an agent, MINIA, (monkey-intra-nuclear-inclusion agent), from monkey tissues which is now recognized to be the same as measles virus. Infection of all primates with this virus is a result of contact with man. Newly captured animals do not have any evidence of infection and subsequently convert to seropositive during captivity. Generally this infection is mild with overt disease a rare occurrence. However, the marmoset may be exquisitely sensitive as infection of these animals with measles virus does produce a widespread and fatal infection.[74] The full spectrum of simian susceptibility to this virus is not known.

Other myxoviruses are known to infect simians but generally produce inapparent or mild disease. It is highly probable that virus strain and dosage are important. CCA (chimpanzee coryza agent) was first isolated from a chimpanzee with respiratory illness by Morris et al.[75] It was later demonstrated by Chanock et al.[76] that this virus is responsible for respiratory disease of infants (RS-respiratory syncytial) and is a human rather than a simian virus. Mumps virus was reported by Block [77] as capable of producing clinical parotitis in monkeys but this apparently is a rare occurrence. Likewise, influenza (at least certain strains) may produce mild respiratory disease in monkeys.[78–79]

Herpesviruses—brief mention should be made of infection of nonhuman primates with two human herpesviruses, the Epstein-Barr virus (EBV) recovered from human lymphomas [80] occurring in Africa (Burkitt's tumor) and *H. hominis* which is one of the more prevalent of human viruses.

There is no evidence of overt disease occurring in simians as a result of natural or even experimental infection with EBV.[81] However, seroepidemiological studies have demonstrated that a number of simian species do possess antibodies to this agent.[81–82] Recently we have demonstrated that there is a phylogenetic susceptibility, again as indicated by the presence of antibody, to EBV among the monkeys and apes (unpublished data). This may be interpreted as suggesting that only ape and higher monkey may be used successfully for experimental studies with this virus or perhaps may even be responsive to natural infection.

Recent experimental studies with *H. hominis*, type 2 by London et al.[83] and Nahmias et al.[84] have demonstrated that the capuchin monkey *(Cebus apella, C. albifrons)* was susceptible to vaginal infection with this virus. The rhesus *(M. mulatta)* and squirrel monkey *(Saimiri sciureus)* did not develop any genital lesions. In our laboratory the susceptibility of the capuchin to *H. hominis* was confirmed. The baboon *(Papio cynocephalus)* was also found to be resistant but the marmoset *(Saguinus oedipus* and *S. fuscicollis)* was highly susceptible with death resulting unless small doses of virus (10–100 $TCID_{50}$) were employed.[85]

SUMMARY

Some 20 years have passed since monkeys have been introduced into the laboratory for large scale biomedical experimentation other than for behavioral studies. The finding that monkeys and apes harbor numerous viruses apparently still comes as a surprise to many individuals who conceive of these animals as nothing more than "test tubes." The significance of these simian viruses has gained some recognition if only from the economic loss engendered by their presence in tissues intended for vaccine production. Thus, much loss of culture material has resulted from contamination by one or another of these simian viruses. It is difficult to assess the true value of these events because there are so few data available. Animals, particularly primates, very rarely show any overt evidence of illness. Many times illness goes unnoticed until the animal is unexpectedly found dead in its cage. Criteria such as loss of appetite or hair changes may not occur or are often not noticed and the animal will die with its cheek pouches full of food. The range of possible effects such events may have

upon interpretation of data is without question manifold. The relationship of these agents to overt human and colony health has been stressed throughout this discussion.

A number of the simian virus isolates, mostly adenoviruses (SA7, SV20, SV33, SV34, SV37, SV38) and SV40 (a papovavirus) have been shown to be oncogenic in newborn hamsters and other rodents. It should be emphasized that no known tumors have ever been attributed to any of these agents in man or other primates. SV40 has been given to thousands of recipients of both live and dead poliovirus vaccine as an unknown contaminant. Many of these individuals have responded with development of antibody not only to the poliovirus strains but to SV40 as well.

In conclusion, numerous viruses are now recognized as persisting within host tissues for long periods of time even to the duration of the animal's life. In the course of this discussion, we have alluded to certain of these as found within simian tissues, for example, herpesviruses. Obviously, there are others! We have mentioned the ability of herpesviruses to produce devastating disease when invading another animal species. It appears that this virus group has another capability, that of becoming quiescent and producing latent infections. Immunosuppression frequently results in the activation of these viruses: herpes simplex, cytomegalovirus, varicella in man and other herpesviruses in different animals. Activation of these viruses is frequently seen in patients with immunologic deficiencies which would strongly suggest that the immune mechanism may be an important factor in maintaining the latent state of these viral agents.

One may only speculate at this time regarding the presence of all these simian viruses within the tissues and body cavities of monkeys and apes. The vast majority of these organisms do not appear to be related to anything specific—or even nonspecific. It is contrary to the orderly nature of biology to maintain a system of little purpose. Are these organisms simply without purpose or are they, like those involved in producing latent infections, awaiting their opportunity to become overt and produce disease —or perhaps cancer?

REFERENCES

1. Hull, R., Minner, J. R., and Smith, J. W.: New viral agents recovered from tissue culture of monkey cells. I. Origin and properties of cytopathogenic agents SV1, SV2, SV4, SV5, SV6, SV11, SV12 and SV15. Amer. J. Hyg., 63:204–215, 1956.
2. Hull, R. M., and Minner, J. R.: New viral agents recovered from tissue cultures of monkey kidney cells. II. Problems of isolation and identification. Ann. N. Y. Acad. Sci., 67:413–423, 1957.
3. Hull, R., Minner, J. R., and Mascoli, C. C.: New viral agents recovered from tissue cultures of monkey kidney cells. III. Recovery or additional agents both from cultures of monkey tissues and directly from tissues and excreta. Amer. J. Hyg., 68:31–44, 1958.
4. Malherbe, H. and Harwin, R.: Seven viruses isolated from the vervet monkey. Brit. J. Exp. Path., 38:539–541, 1957.
5. Hull, R. N.: The Simian Viruses. Virology Monograph #2, Springer-Verlag, New York, Inc. pp. 1–66, 1968.
6. Hsiung, G. D.: Latent virus infections in primate tissues with special reference to simian viruses. Bact. Rev., 32:185–205, 1968.
7. Hsiung, G. D.: Recent advances in the study of simian viruses. Ann. N. Y. Acad Sci., 162:483–498, 1969.
8. Kalter, S. S., and Heberling, R. L.: Comparative virology of primates. Bact. Rev., 35:310–364, 1971.
9. Kalter, S. S.: Virus diseases of nonhuman primates. Sci. Pub., 182:86–99, 1969.
10. Kalter, S. S.: Nonhuman primates in viral research. Ann. N. Y. Acad. Sci., 162:499–528, 1969.
11. Haas, R.: Experimentelle Infektionen von Cercopithecus aethiops mit dem Erreger des Frankfurt-Marburg-Syndroms (FMS). J. Embryol. Exp. Morphol., 20:210–220, 1968.
12. Kissling, R. E., Murphy, F. A., and Henderson, B. E.: Marbury virus. Ann. N. Y. Acad. Sci., 174: 932–945, 1970.
13. Kissling, R. E., Robinson, R. Q., Murphy, F. A., and Whitfield, S. G. Agent of disease contracted from green monkeys. Science, 160:888–890, 1968.
14. Kafuko, G. W., Henderson, B. E., Williams, M. C. and Kissling, R. E.: Investigations in Uganda relating to the Marburg agent. *in* "Infection and Immunosuppression in Primates." Balner, H. and Beveridge, W. I. B., eds., Munksgaard, Copenhagen, pp. 45–48, 1970.
15. Kalter, S. S.: A serological survey of primate sera for antibody to the Marburg virus. *in* "Proc. Symposium on Marburg Virus Disease." Martini, G. A. and Siegert, R., eds., Springer-Verlag, Berlin, pp. 177–187, 1971.
16. Kalter, S. S., Ratner, J. J., and Heberling, R. L.: Antibodies in primates to the Marburg virus. Proc. Soc. Exp. Biol. Med., 130:10–12, 1969.

17. Malherbe, H., and Strickland-Cholmlay, M.: Studies on the Marburg virus. *in* "Proc. Symposium on Marburg Virus Disease" Martini, G. A. and Siegert, R., eds., Springer-Verlag, Berlin, pp. 188–194, 1971.
18. Slenczka, W., Wolff, G., and Siegert, R.: A critical study of monkey sera for the presence of antibody against the Marburg virus. Amer. J. Epidem., 93:496–505, 1971.
19. Arita, I., and Henderson, D. A.: Smallpox and monkeypox in non-human primates. Bull. Organ. Mond. Sante., 39:277–283, 1968.
20. Gispen, R., Verlinde, J. D., and Zwart, P.: Histopathological and virological studies on monkeypox. Arch. ges. Virusforsch., 21:205–216, 1967.
21. Hahon, N.: Smallpox and related poxvirus infections in the simian host *(Papio cynocephale)*. Bact. Rev., 25:459–476, 1961.
22. McConnell, S. J., Herman, Y. F., Mattson, D. E., Erickson, L.: Monkey pox disease in irradiated cynomolgus monkeys. Nature, 195:1128–1129, 1962.
23. Prier, J. E., and Sauer, R. M.: A pox disease of monkeys. Ann. N. Y. Acad. Sci., 85:951–959, 1960.
24. von Magnus, P., Anderson, E. K., Petersen, K. B., and Birch-Anderson, A.: A pox-like disease in cynomolgus monkeys. Acta. path. microbiol. scand., 46:156–176, 1969.
25. Prier, J. E., Sauer, R. M., Malsberger, R. G., and Sillaman, J. M.: Studies on a pox disease of monkeys. II. Isolation of the etiologic agent. Amer. J. Vet. Dis., 21:381–384, 1960.
26. Sauer, R. M., Prier, J. E., Buchanan, R. S., Creamer, A. A., and Fegley, H. C.: I. Studies on a pox disease of monkeys. I. Pathology. Amer. J. Vet. Res., 21:377–380, 1960.
27. Peters, J. C.: An epizootic of monkey pox at Rotterdam Zoo. Int. Zoo Yearb. 6:274–275, 1966.
28. Peters, J. C.: Eine "Monkey-Pox" Enzootie im Affenhaus des Tiergartens "Blijdorp." (A "monkey-pox" zoonosis in the monkey-house of the "Blijdorp" zoological gardens. Kleintier-Praz., 11:65–71, 1966.
29. Raghavan, R. S., and Khan, G. A.: An outbreak of pox in chimpanzees. Indian Vet. J., 45:75–76, Jan. 1968.
30. Milhaud, C., Klein, M., and Virat, J.: Analyse d'un cas de variole du singe (monkey pox) chez le chimpanze. *(Pan troglodytes)*. Exp. Anim., 2:121–135, 1969.
31. Human infection with monkeypox virus, Liberia and Sierra Leone.: Morbid. Mortal. Wkly Rep., 20:65–72, 1971.
32. McConnell, S., Herman, Y. F., Mattson, D. E., Huxsoll, D. L., Lang, C. M., Yager, R. H.: Protection of rhesus monkeys against monkeypox by vaccinia virus immunization. Amer. J. Vet. Res., 25:192–195, 1964.
33. McConnell, S., Hickman, R. L., Wooding, W. L., Jr., and Huxsoll, D. L.: Monkeypox: experimental infection in chimpanzee *(Pan satyrus)* and immunization with vaccinia virus. Amer. J. Vet. Res., 29: 1675–1680, 1968.
34. Heberling, R. L., and Kalter, S. S.: Induction, course, and transmissibility of monkeypox in the baboon *(Papio cynocephalus)*. J. Infect. Dis., 124: 33–38, 1971.
35. Espana, C.: A pox disease of monkeys transmissible to man. *in* "Medical Primatology." 2nd Conf. Exp. Med. & Surg. in Primates, New York, Sept. 7–12, pp. 694–708, 1969. Goldsmith, E. I., and Moor-Jankowski, J., eds. Karger Basel/New York, 1971.
36. Bearcroft, W. G. C., and Jamieson, M. F.: An outbreak of subcutaneous tumors in rhesus monkeys. Nature, 182:195–196, 1958.
37. Back, N., Ambrus, J. L., and Kalter, S. S.: The use of the primate in oncogenic virus studies. *in* "The Baboon in Medical Research" Vagtborg, H., ed. 1:421–429, University of Texas Press, Austin, 1965.
38. Ambrus, J. L., and Strandstrom, H. V.: Susceptibility of Old World monkeys to Yaba virus. Nature, 211:876, 1966.
39. Casey, H. N., Woodroff, J. M., and Butcher, W. I.: Electron microscopy of a benign epidermal pox disease of rhesus monkeys. Amer. J. Path., 51:431–446, 1967.
40. Holmes, A. W., Caldwell, R. G., Dedmon, R. E., and Deinhardt, F.: Isolation and characterization of a new herpes virus. J. Immunol., 92:602–610, 1964.
41. Holmes, A. W., Devine, J., Nowakowski, E., and Deinhardt, F.: The epidemiology of a herpes virus infection of New World monkeys. J. Immunol., 96: 668–671, 1966.
42. Melnick, J. L., Midulla, M., Wimberly, I., Barrerra-Oro, J. G., and Levy, B. M.: A new member of the herpesvirus group isolated from S. American marmosets. J. Immunol., 92:596–601, 1964.
43. Melendez, L. V., Daniel, M. D., Hunt, R. D., and Garcia, F. G.: An apparently new herpesvirus from primary kidney cultures of the squirrel monkey *(Saimiri sciureus)*. Lab. Anim. Care, 18:374–381, 1968.
44. Melendez, L. V., and Hunt, R. D.: Current views on herpes-T infection in South American monkeys. Proc. 5th Pan American Cong. Vet. Med. & Zootechnic, 1966, Caracas, Venezuela.
45. Melendez, L. V., Hunt, R. D., Garcia, F. G., and Trum, B. F.: A latent herpes-T infection in *Saimiri sciureus* squirrel monkey). *in* "Some Recent Developments in Comparative Medicine" Fiennes, R. N. T. W., ed. Academic Press, London, 1966, pp. 393–397.
46. Lennette, E. N.: Workshop on viral diseases which impede colonization of nonhuman primates. Nat'l. Center for Primate Biology, Univ. of Calif. at Davis, May 22–24, 1968.
47. Melendez, L. V.: Herpes viral diseases in New World primates. Sci. Pub., 182:103–106, 1969.

48. Melendez, L. V., Espana, L., Hunt, R. D., Daniel, M. D., and Garcia, F. G.: Natural herpes simplex infection in the owl monkey *(Aotus trivirgatus)*. Lab. Anim. Care, 19:38–45, 1969.

49. Melendez, L. V., Garcia, F. G., Fraser, C. E. O., Hunt, R. D., and King, N. W.: *Herpesvirus saimiri*. I. Further characterization studies of a new virus from the squirrel monkey. Lab. Anim. Care, 19: 372–377, 1969.

50. Melendez, L. V., Hunt, R. D., Daniel, M. D., Fraser, C. E. O., Garcia, F. G., and Williamson, M. E.: Letal reticuloproliferative disease induced in *Cebus albifrons* monkeys by *Herpesvirus saimiri*. Int. J. Cancer, 6:431–435, 1970.

51. Melendez, L. V., Hunt, R. D., Daniel, M. D., and Garcia, F. G.: Natural herpes simplex infection in owl monkeys *(Aotus trivirgatus)*. Fed. Proc., 27: 664, 1968.

52. Melendez, L. V., Hunt, R. D., Garcia, F. G., and Fraser, C. E. O.: *Herpesvirus saimiri*. II. An experimentally induced malignant lymphoma in primates. Lab. Anim. Care, 19:378–386, 1969.

53. Murphy, B. L., Maynard, J. E., Krushak, D. H., and Fields, R. M.: Occurrence of a carrier state for *Herpesvirus tamarinus* in marmosets. Appl. Microbiol., 21:50–52, 1971.

54. Clarkson, M. J., Thorpe, E., and McCarthy, K.: A virus disease of captive monkeys *(Cercopithecus aethiops)* caused by a new herpesvirus. Arch. ges. Virusforsch., 22:219–234, 1967.

55. McCarthy, K., Thorpe, E., Laursen, A. C., Heymann, C. S., and Beale, A. J.: Exanthematous disease in patas monkeys caused by a herpes virus. Lancet, 2:856–857, 1968.

56. Kalter, S. S.: Serologic surveys. *in* "Pathology of Simian Primates" Part II Fiennes, R. N. T. W. ed. S. Karger, Basel, 1972, pp. 469–496.

57. Kalter, S. S., Fuentes-Marins, R., Crandell, R. A., Rodriguez, A. R., and Hellman, A.: Virus complement-fixation and neutralization studies on sera from the normal baboon *(Papio doguera)*. J. Bact., 87:744–746, 1964.

58. Kalter, S. S., Ratner, J., Kalter, G. V., Rodriguez, A. R., and Kim, C. S.: A survey of primate sera for antibodies to viruses of human and simian origin. Amer. J. Epidem., 86:552–568, 1967.

59. Kalter, S. S., and Jeffries-Klitch, H.: Yellow fever vaccination of primates. Amer. J. Trop. Med. Hyg., 18:466–469, 1969.

60. Work, T. H., and Napido, H.: Kyasanur forest disease. A new virus disease in India. Summary of preliminary report of investigations of the Virus Research centre on an epidemic disease affecting forest villagers and wild monkeys of Shimoga District, Mysore, Ind. J. Med. Sci., 11:340, 1957.

61. Shah, K. V.: Experimental infection of lactating monkeys with Kyasanur forest disease virus. Acta. Virol., 9:71–75, 1965.

62. Hillis, W. D.: An outbreak of infectious hepatitis among chimpanzee handlers at a United States Air Force Base. Amer. J. Hyg., 73:316–328, 1961.

63. Bearcroft, W. G. C.: Hepatitis in African monkeys. Nature, 197:806–807, 1963.

64. Bearcroft, W.: Histological studies on the livers of West African primates inoculated with infectious hepatitis. J. Pathol. Bact., 88:511–519, 1964.

65. Bearcroft, W. G. C.: Studies on the transmission of the agent of infectious hepatitis to patas monkeys. J. Med. Microbiol., 1:1–21, 1968.

66. Bearcroft, W. G. C.: Further studies on the transmission of the agent of infectious hepatitis to patas monkeys. Brit. J. Exp. Pathol., 50:56–65, 1969.

67. Bearcroft, W. G. C.: Susceptibility of patas monkeys to re-infection with infectious hepatitis. Brit. J. Exp. Pathol., 50:327–330, 1969.

68. Deinhardt, F.: Hepatitis in subhuman primates and the hazards to man. *in* "Infection and Immunosuppression in Subhuman Primates" Balner, H. and Beveridge, W. I. B., eds., Munksgaard, Copenhagen, 1970, pp. 55–63.

69. Deinhardt, F., Holmes, A. W., Capps, R. B., and Popper, H.: Studies on the transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passages, and description of liver lesions. J. Exp. Med., 125:673–688, 1967.

70. Melnick, J. L., Parks, W. P., Deinhardt, F., Holmes, P. W., and Wolfe, L. G.: Hepatitis in marmosets. J. Infect. Dis., 121:351–354, 1970.

71. Melnick, J. L.: Discussion of transmission of human viral hepatitis to nonhuman primates. *in* "Perspectives in Virology" Vol. VI. Pollard, M. ed., Academic Press, New York, 1968, pp. 99–103.

72. Blumberg, B. S., Sutnick, A. I., and London, W. T.: Hepatitis and leukemia: their relation to Australia antigen. Bull. N. Y. Acad. Med., 44: 1566–1586, 1968.

73. Ruckle, G.: Studies with the monkey-intranuclear inclusions agent (MINIA) and foamy agent derived from spontaneously degenerating monkey kidney cultures. I. Isolation and tissue culture behavior of the agents and identification of MINIA as closely related to measles virus. Arch. ges. Virusforsch., 8:139–166, 1958.

74. Levy, B. M., and Mirkovic, R. R.: An epizootic of measles in a marmoset colony. Lab. Anim. Sci., 21: 33–39, 1971.

75. Morris, J. A., Blount, R. E., Jr., and Savage, R. E.: Recovery of cytopathogenic agent from chimpanzees with coryza. Proc. Soc. Exp. Biol. Med., 92:544–549, 1956.

76. Chanock, R., Roizman, B., and Myers, R.: Recovery from infants with respiratory illness of virus related to chimpanzee coryza agent (CCA). I. Isolation, properties, and characterization. Amer. J. Hyg., 66:281–290, 1957.

77. Bloc, O., Jr.: Specificity of lesion of experimental mumps. Amer. J. Pathol., 13:939–944, 1937.

78. Heberling, R. L., and Kalter, S. S.: Persistence and spread of influenza virus (A2/Hong Kong) in normal and poly I.C. treated baboons *(Papio cynocephalus)*. Proc. Soc. Exp. Biol. Med., 135:717–723, 1970.
79. Saslaw, S., Wilson, H. E., Doan, L. A., Woolpert, O. C. and Schwab, J. L.: Reactions of monkeys to experimentally induced influenza virus A infection. An analysis of the relative roles of humoral and cellular under conditions of deficient nutrition. J. Exp. Med., 84:113–125, 1946.
80. Burkitt, D.: A sarcoma involving the jaws in African children. Brit. J. Surg., 46:218–223, 1958.
81. Gerber, P., and Birch, S. M.: Complement-fixing antibodies in sera of human and nonhuman primates to viral antigens derived from Burkitt's lymphoma cells. Proc. N. A. S. at Cadci. 58:478–484, 1967.
82. Gerber, P., and Rosenblum, E. N.: The incidence of complement-fixing antibodies to herpes simplex, and herpes-like viruses in man and rhesus monkeys. Proc. Soc. Exp. Biol. Med., 128:541–546, 1968.
83. London, W. T., Catalano, L. W., Jr., Nahmias, A. J., Fuccillo, D. A. and Sever, J. L.: Genital *Herpesvirus hominis* type 2 infection of monkeys. Obstet. & Gynec., 37:501–509, 1971.
84. Nahmias, A. J., London, W. T., Catalano, L. W., Fuccillo, D. A., Sever, J. L., and Graham, C.: Genital *Herpesvirus hominis* type 2 infection: an experimental model in cebus monkeys. Science, 171: 297–298, 1971.
85. Kalter, S. S., Felsburg, P. J., Heberling, R. L., Nahmias, A. J., and Brack, M.: Experimental *Herpesvirus hominis* type 2 infection in nonhuman primates. Proc. Soc. Exp. Biol. & Med., 139:964–968, 1972.

TAXONOMIC NOTES

1. No names or combinations thereof are proposed as new names. The nomenclature generally follows Napier and Napier (1967), except where noted.
2. Animals identified as *Cercocebus atys* and *C. fuliginosus* are listed under *C. torquatus*.
3. Listed under *Cercopithecus mitis* are also animals reported as *C. albogularis*.
4. Listed under *Macaca nigra* are also animals reported as *M. maurus, M. hecki,* and *M. tonkeanus*.
5. Listed under *Papio cynocephalus* are also animals reported as *Papio anubis, P. doguera, P. papio,* and *Papio* sp.
6. *Lemur fulvus* was listed on the questionaire. *L. macaco* was not. As described in Napier and Napier (1967) they are now considered conspecific under the latter name.
7. *Tupaia* was not listed on the questionnaire. It is not known whether some respondents did not list tree shrews they were maintaining because they considered them to be insectivores, not primates.

DISCUSSION

Harry M. Glass, Harvard University: Do you have any knowledge of what role karyotypes might have in prevention of your various herpes diseases?

Dr. Kalter: I'm sorry, I'm not sure I know what you mean by karyotypes in this context.

Dr. Glass: (Inaudible)

Dr. Kalter: No, I'm sorry.

Incidentally, Dr. Pollard, I might mention that monkey pox, which is innocuous, as we indicated, in conventional animals, is highly devastating in germfree monkeys.

DIROFILARIA IMMITIS: A REVIEW OF PRESENT KNOWLEDGE

G. Pacheco*

Dirofilaria immitis, the dog heartworm, is highly endemic throughout the southeastern portion of the United States. Although its distribution in the rest of the country is not as well documented, recent reports indicate that it is prevalent. In fact, some reports document extremely high focalized incidence. Adult *D. immitis*, commonly found in the right ventricle and pulmonary arteries, shed their embryos (microfilariae—MF) into the blood stream. The MF are ingested by mosquitoes, migrate to the Malpighian tubules and develop intracellularly for 12 to 16 days before they are infective for a second host. In dogs pathological changes ascribed to infection with *D. immitis* are noted in the heart and pulmonary arteries. Additionally, immunologic damage of glomeruli has been reported in kidneys of dogs with large numbers of MF in circulation. In man the "coin-type" of lesion seen radiologically in lung is commonly associated with dead juvenile *D. immitis*. Although treatment of infections with heartworm in the dog remains primitive—organic arsenicals to destroy the adults and dithiazinine iodide for the MF—prophylaxis is now well established. Daily use of diethylcarbamazine in sentry dogs maintained by the Air Force, as well as in colonies privately maintained for hunting, has resulted in complete protection against this parasite.

INTRODUCTION

Taxonomically, *Dirofilaria immitis* is one of a very large group of nematodes known as filariae. Most terrestrial vertebrates are parasitized by at least one of these nematodes which have in common the requirement of a blood-sucking arthropod as a combination vector—intermediate host. Adult worms of this group of nematodes are commonly found in subcutaneous tissues or in the body cavities and, exceptionally as in the case of the dog heartworm, directly in the circulatory system. Reproductive products—microfilariae (MF)—are in the bloodstream or in skin and are ingested by the arthropod when it feeds.

*U. S. Department of Health, Education, and Welfare; National Institutes of Health; National Institute of Allergy and Infectious Diseases; Laboratory of Parasitic Diseases, Bethesda, Maryland 20014.

Adult male and female *D. immitis* live in the right ventricle and pulmonary arteries of dogs. The females are approximately 25 cm long and about 1 mm in diameter. The males are shorter, approximately 16 cm in length, but have the same diameter. Although they are commonly found in the right ventricle, they may also be in the pulmonary arteries, particularly those of the diaphragmatic lobes of the lung. The impression that one has from this localization is that the worms move at will from the heart to the arteries and back. In this location the adult females shed the MF directly into the bloodstream. The MF, which are actively moving embryos measuring approximately 300 μ in length by 6 to 7 μ in diameter are picked up from the bloodstream by mosquitoes when these take a blood meal. The MF migrate out of the stomach and penetrate the Malpighian tubules of the mosquito. They take an intracellular position in the Malpighian tubules and under optimal conditions develop for at least two weeks. Development in the Malpighian tubules of the mosquito involves extensive growth, molting and reorganization, arriving finally at a third stage larva. This larva is approximately 1 mm in length by 25 μ in diameter. It breaks out of the Malpighian tubules and migrates to the head of the mosquito into the sheath surrounding the proboscis. Thus, when the mosquito takes a blood meal the sheath is bent, ruptured and the infective-stage larvae deposited right next to the puncture wound made by the stylets of the biting mosquito. When the stylets are withdrawn, the larvae enter the vertebrae host through this puncture wound and begin their development to adult worms.

The development from third-stage infective larvae to adult worms requires approximately six months. From two to two and one-half months of this time the worms are in sub-

cutaneous tissues. The first week involves adjustment of the parasite to a completely new and different environment, the vertebrae host. The end of this period of adjustment is marked by a molt occurring seven to ten days after the larvae have penetrated into the subcutaneous tissues of the dog. The larvae during this time migrate quite freely in the subcutaneous tissues insinuating themselves between cells and, characteristically, not evoking a cellular response. Migration to the heart is first seen about 70 days after infection and by 90 days worms can no longer be recovered from the subcutaneous tissues. Although the migration is to the heart, it should be noted that many of these juvenile worms are recovered from the distal portions of the pulmonary arterial tree and the pathological changes noted in pulmonary arteries, to be discussed later, suggest that they spend most of their time in this location. The reproductive system in these worms becomes functional early in the fourth month of the infection and fully-developed MF are present in the uteri and vagina of the worms at six months. Thus, 25 to 30 weeks after infection MF can be detected in the peripheral circulation.[1]

Pathological changes attributed to *Dirofilaria immitis* can be considered under two headings: changes attributable to developing larvae or adult worms and changes associated with the MF. The most obvious problem associated with adult worms is mechanical. The presence of these large worms in the right ventricle and pulmonary arteries blocks and embarasses circulation and may result in hypertrophy of the heart, fibrosis of valves and chronic passive congestion of vital organs. In general the severity of these changes is directly related to the number of worms present. Secondly, but equally important is the fibrosis of the endothelium of the pulmonary arteries which can be quite extensive. Until recently it was believed that this fibrosis was elicited by mechanical insult to the endothelium, but studies at Brooks Air Force Base, Texas, suggest strongly that this may be an allergic reaction to products of the worms.[2]

Although documentation of antigenic differences between adult worms and MF has been available for a number of years[3,4,5,6] and disease specifically attributable to MF has been extensively documented in human filariases, it is only in recent years that disease associated with MF of *D. immitis* in the dog has been investigated. Casey documented extensive damage to glomeruli in dogs infected with *D. immitis*.[2] He indicated that this damage could be associated with the deposition of antigen-antibody-complement complexes.

Although pathological changes associated with developing third- or fourth-stage larvae in the vertebrae have not been considered damaging to the host, effects of sensitization resulting from the extensive tissue invasion have not been studied.

It is well known, and extensively documented, that *D. immitis* is highly endemic throughout the southeastern portion of the United States. However, there is a lack of documentation on the incidence of the parasite in other parts of the country. Some recent reports, such as that of Mallack et al. of dogs maintained by three hunt clubs in Maryland, suggest that the incidence of *D. immitis* is over 50 percent.[7] However, surveys done in dogs that were not housed in the same relatively restricted quarters suggest that in fact the incidence is much lower, probably of the order of 5 percent. There are few surveys of large populations of dogs in the midwestern region of the country, but in these the reported incidence of heartworm is relatively low, less than 5 percent. In the western states the incidence of *D. immitis* is indeed very low. There are occasional reports of dogs infected with the parasite, but these are so rare that one would suspect importation of the dog or close association with an imported dog.

A major problem associated with surveys for *D. immitis* is the widespread distribution of the related filaria, *Dipetalonema reconditum*. It was only in 1956 that Newton and Wright recognized that a second filarial worm commonly parasitized dogs in the United States.[8] Although MF of this parasite usually are not abundant, when observed they must be differentiated from MF of *D. immitis*. In some instances it is clear that the investigators involved in the survey were carefully separating the two species,[9] but in others it is quite evident that differentiation between *D. immitis* and *D. re-*

conditum was not done. Thus, at this time it is difficult to determine the precise prevalence of this parasitic infection in dogs throughout most of the U. S. However, in the southeastern region of the U. S. the prevalence is high: 20 to 60 percent of dogs surveyed. Some careful surveys, such as the one mentioned earlier in dogs maintained by hunt clubs[7] indicate clearly that whenever dogs are housed in close proximity to one another, there is a real danger that a hyperendemic focus will be established. However, existence of such a hyperendemic focus does not mean that the incidence in the surrounding region will equal that of the focus.

While it is well established that *D. immitis* is transmitted by mosquitoes and laboratory transmission of the parasite is routinely done with several species of either anopheline or culicine mosquitoes, there is no information on the natural vectors of the parasite in any part of the country. Ludlam et al. tested a number of wild-caught species of mosquitoes to determine their ability to transmit *D. immitis* and found that ten species common to Maryland would transmit the infection in the laboratory.[10] There is no information on whether these same species can serve as vectors in nature. Thus, control of the diseases directed at the vector must await solid documentation on which mosquito(s) really serve as vectors.

Diagnosis of infections with *D. immitis* has been based on finding MF in blood but there are a number of problems associated with this. First, if MF are seen, how is one to be certain that there are *D. immitis* and not *D. reconditum?* Some of the early studies emphasized the presence of a "button hook" tail on MF or *D. reconditum*[8,11] but it has since been recognized as an unreliable feature. Although the length and diameter of these MF are substantially different and, if measured, will serve to readily distinguish the two species, the histochemical differentiation of the two species provided by Chalifoux and Hunt has given practitioners and people who work with these filariae greater confidence in their ability to distinguish between the two species.[12]

The second problem that has troubled many people who must evaluate the health status of dogs is that when MF are not present in large numbers they may be difficult to detect. Although somewhat more cumbersome than examination of a simple blood smear, the efficiency of the concentration technics that have been developed is such that only a very small proportion of dogs with microfilaremia will be misdiagnosed.[13,14] While diagnosis of infection when there is microfilaremia is relatively simple, it is a common experience to obtain a dog free of MF only to find that in fact adult or juvenile worms are present in the heart. Diagnosis of these pre-patent or non-patent infections is at present not feasible. Several reports have indicated that antibodies are readily detectable in sera from dogs during the prepatent period of the infection, however, all of these studies were done with experimentally infected dogs in which it was possible to study antibody levels before and after infection, thus a titer differential could be determined.[15,16,17] Secondly, when currently available serological tests have been used with sera from noninfected controlled dogs housed indoors in areas of low endemnicity, there has been a high incidence—over 20 percent—in which significant levels of antibody have been detected. Thus, currently available serological tests for filariasis suffer from excess cross-reactivity. These tests are useful to indicate the extent or degree of prepatent infection in a geographic region, i.e., as epidemiological tools, but they cannot be relied on to provide a diagnosis for individual dogs.

Recently, purified extracts of *D. immitis* have been used in intradermal tests in attempts to detect prepatent infections.[6] As with serological tests the proportion of false positive reactions is approximately 30 percent. Thus, again, there are no reliable tests at present that can be used to indicate presence of *D. immitis* in a dog before MF are detectable in blood.

Management of heartworm infections has changed significantly during the past few years. Emphasis in the past was only on treatment once the infection was established, but at present emphasis is on chemophophylaxis with diethylcarbamazine. Studies in Japan as well as in the United States clearly demonstrated that chemoprophylaxis is entirely feasible in dogs maintained in colonies or groups in which the

drug can be mixed with feed or given orally daily until two months after the end of the infectious season.[16,18,19]

Clearance of MF is at present achieved with the cyanine dye, dithiazine iodide, and treatment of the adult worms is with organic arsenicals or organophosphates. The latter have proved very effective but must be used with great care because of the danger of other commonly used cholinesterase inhibitors, such as those in flea collars.

Although methods of diagnosis of infections with *D. immitis* have not improved greatly, the availability of inexpensive chemoprophylaxis, which can be readily administered where large groups of dogs are maintained, provides a much more optimistic outlook for heartworm-free dogs that can be used in research.

SUMMARY

Although *D. immitis* is prevalent in most of the United States, it is most prevalent throughout the southeastern region of the country. Currently used technics for concentration of blood can detect essentially all infections with microfilaremia, but as microfilariae are absent during prepatency, as well as in a small percentage of infected dogs, examination of blood cannot provide diagnosis of all infections. False-positive reactions are common in presently-available immunological tests for heartworm. Thus the principal utility of these tests is epidemiological, not diagnosis of individual cases. There has been little change in the treatment of established heartworm infections, but the prophylactic use of diethylcarbamazine provides a method by which colonies of dogs can be maintained free of heartworm.

REFERENCES

1. Orihel, T. C.: Morphology of the larval stages of *Dirofilaria immitis* in the dog. J. Parasitol., 47:251–262, 1961.
2. Casey, H.: Pathological changes in kidneys of dogs infected with *Dirofilaria immitis*. Second University of Florida Symposium on Canine Heartworm Disease, 1971.
3. Franks, M. B.: Specific soluble antigen in the blood of filarial patients. J. Parasitol., 32:400–406, 1946.
4. Beaver, P. C., Pacheco, G. and Wong, M. M.: Hypersensitivity phenomena with special reference to filariasis. Second Medical Conference on Parasitic Diseases, SEATO Medical Laboratories, 1966.
5. Wong, M. M.: Studies on microfilaremia in dogs. II. Levels of microfilaremia in relation to immunologic responses of the host. Am. J. Trop. Med. Hyg., 13: 66–77, 1964.
6. Ah, H. S., Peckham, J. C., Mitchell, F. E., and Thompson, P. E.: Studies on *Dirofilaria immitis* infections in dogs relative to immunizations and antigen-antibody interactions. Second University of Florida Symposium on Canine Heartworm Disease, 1971.
7. Mallack, J., Sass, B. and Ludlam, K. W.: *Dirolaria immitis* in hunting dogs from an area in Maryland. J.A.V.M.A. 159:177–179, 1971.
8. Newton, W. L. and Wright, W. H.: The occurrence of a dog filariid other than *Dirofilaria immitis* in the United States. J. Parasitol., 42:246–258, 1956.
9. Thrasher, J. P. and Clanton, J. R., Jr.: Epizootiologic observations on canine filariasis in Georgia. J.A.V.M.A., 152:1517–1520, 1968.
10. Ludlam, K. W., Jachowski, L. A., Jr. and Otto, G. F.: Potential vectors of *Dirofilaria immitis*. J.A.V.M.A., 157:1354–1359, 1970.
11. Rothstein, N., Kinnanion, K. E. and Brown, M. L.: Canine microfilariasis in Eastern United States. J. Parasitol., 47:661–665, 1961.
12. Chalifoux, L. and Hunt, R. D.: Histochemical differentiation of *Dirofilaria immitis* and *Dipetalonema reconditum*. J.A.V.M.A., 158:601–605, 1971.
13. Knott, J. I.: A method for making microfilarial surveys on day blood. Trans. Roy. Soc. Trop. Med. Hyg., 33:191–196, 1939.
14. Wylie, J. P.: Detection of microfilariae by a filter technique. J.A.V.M.A., 156:1403–1405, 1970.
15. Pacheco, G.: Progressive changes in certain serological responses to *Dirofilaria immitis* infection in the dog. J. Parasitol., 52:311–317, 1966.
16. Warne, R. J., Tipton, V. J. and Furusho, Y.: Canine heartworm disease in Japan: Screening of selected drugs against *Dirofilaria immitis in vivo*. Am. J. Vet. Res., 30:27–32, 1969.
17. Allain, D. S., Kagan, I. G. and Schlotthauer, J. C.: Serologic studies on dogs experimentally infected with *Dirofilaria immitis*. Second University of Florida Symposium on Canine Heartworm Disease, 1971.
18. Kume, S., Ohishi, I. and Kobayashi, S.: A new approach to prophylactic therapy against the developing stages of *Dirofilaria immitis*. Am. J. Vet. Res., 23:1257–1260, 1962.
19. Warne, R. J., Tipton, V. J. and Furusho, Y.: Canine heartworm disease in Japan: Screening of selected drugs against *Dirofilaria immitis in vivo*. Am. J. Vet. Res., 30:27–32, 1969.

DISEASES ENCOUNTERED DURING THE CONDITIONING OF RANDOM SOURCE DOGS AND CATS

A. E. Palmer*

Random source dogs and cats are used extensively in biomedical research. They originate from a wide variety of sources and represent the surplus products of uncontrolled breeding. Frequently they have undesirable conformation or temperament and are afflicted with diseases.

Between the time of purchase by the dealer and delivery to the research laboratory, random source animals are subjected to several environmental and food changes, contact with sick animals, and exposure to numerous pathogenic agents. A period of conditioning is therefore necessary to allow the animals to overcome or coexist with their pathogenic flora and to adjust to their new environment prior to their introduction into research programs.

This presentation will describe the quarantine and conditioning program for dogs and cats at the NIH and describe some of the disease conditions seen among quarantined animals.

INTRODUCTION

Several hundred thousand dogs and cats are used annually for research in the United States.[1-3] According to data reported by The Institute of Laboratory Animal Resources, 8.4 percent of the cats and 11.7 percent of the dogs used during 1969 were bred specifically for research use. The remaining animals which originated from a variety of sources including pounds, animal auctions, or private kennels were designated "random source." Their background varied from urban to rural, and their experience with pathogenic agents was extensive. The purpose of this report is to describe some of the quarantine procedures of the NIH Animal Center and to review some of the problems encountered in dealing with a highly stressed, hyperexposed and unstable animal population before it is ready to be used in laboratory experimentation.

* National Institutes of Health Division of Research Services Bethesda, Maryland 20014.

NIH ANIMAL CENTER

The NIH purchases dogs from a USDA class B animal dealer who obtains animals primarily from the east-central states. Cats are acquired from a licensed dealer who buys them primarily from dairy farms within a 50 mile radius from his Maryland holding facility. During fiscal year 1971, 87 percent of 2,401 cats and 52.5 percent of 1,837 dogs used at the NIH were random source animals.

The history of the random source dog quarantine program at NIH has been described by Zinn.[4] The canine breeding and blood donor colonies are described by Potkay and Bacher.[5] Dogs and cats are presently conditioned for a 45-day period. Dogs are purchased after 30 days of conditioning by the vendor and held 15 days in the quarantine colony. Cats are purchased in an unconditioned state and quarantined for 45 days.

The National Heart-Lung Institute (NHLI) uses about 87 percent of the dogs, 63 percent of the cats and 95 percent of the canine blood produced. Because the dogs are used primarily in cardiovascular surgical procedures, large, adult dogs, weighing more than 40 pounds, are used almost exclusively.

Facilities

All dog and cat quarantining is performed in a kennel complex located at the NIH Animal Center. A floor plan of this complex is shown in Figure 1. The building provides a central service area which has, in addition to service facilities, 6 animal rooms, each containing 40 stainless steel and aluminum cages. Two wings, each containing 100 animal runs, project perpendicularly from the central service area. The primary animal enclosures conform to the recommendations of the Institute of Laboratory Animal Re-

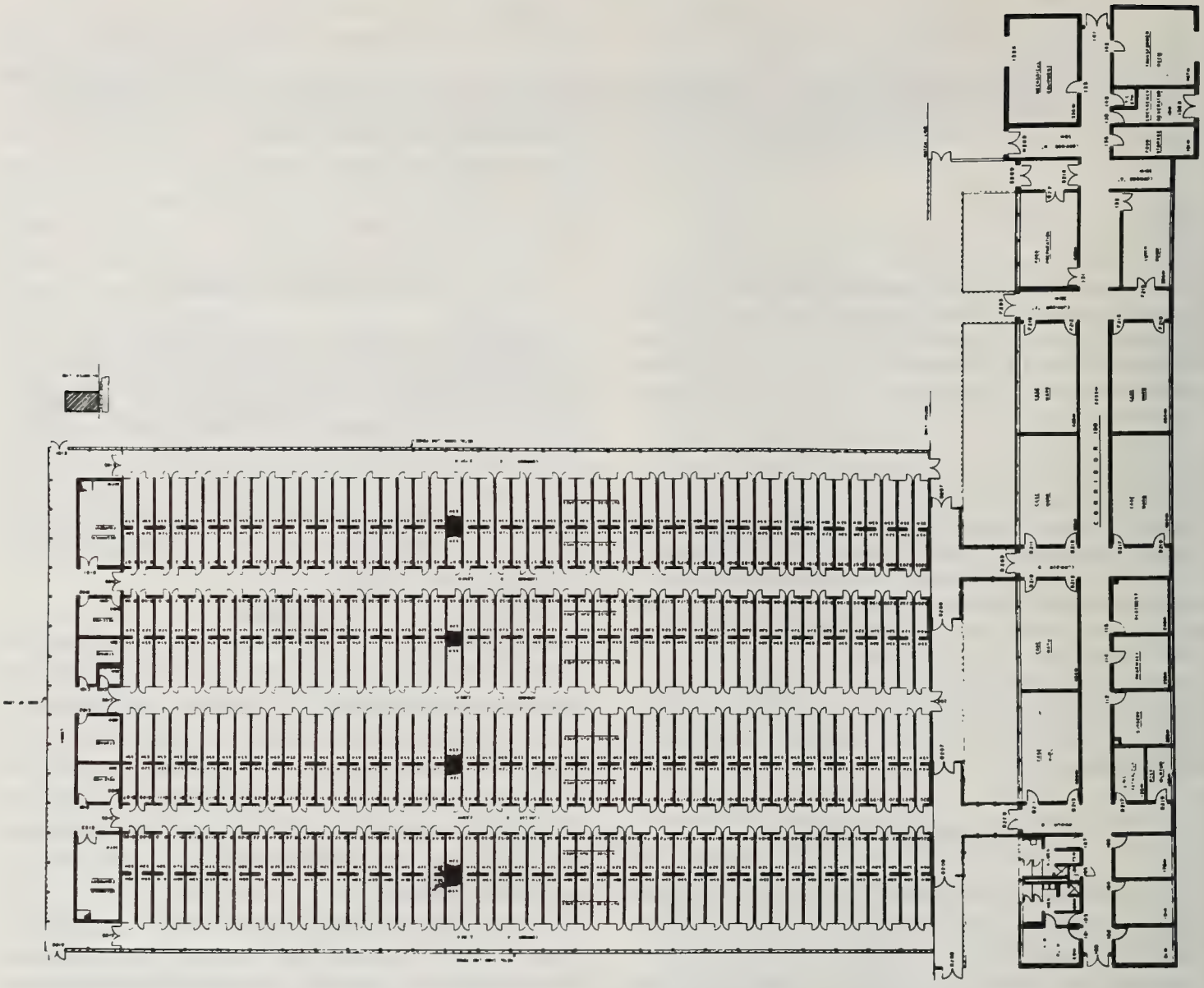


FIGURE 1.—Floor Plan of Kennel Complex at the NIHAC where Random Source Dogs and Cats are held for conditioning.

sources. Dogs and cats are provided space which meets or exceeds the specifications of the USDA.

Random Source Dog Quarantine and Conditioning

All random source dogs undergo 45 days of quarantine and conditioning prior to their release to NIH laboratories. Before January 1970 this was done entirely at NIH facilities. Since then, dogs which are purchased have been preconditioned by the vendor for 30 days. This program change reduced the size of the random source dog colony by about 65 percent, releasing space to permit the expansion of the canine breeding program.

During the 30-day conditioning period at the vendor's facility, the dogs receive immunizations against rabies, canine distemper and hepatitis. They are treated for endo- and ectoparasites, and are fed a diet similar to that used for the NIH dog colony. One to three samples of blood from each dog are examined for the presence of microfilariae.

Upon receipt from the vendor, each dog is given a physical examination by a veterinarian, and a blood sample is examined for the presence of microfilariae. Sick and microfilaria positive animals are rejected; accepted animals are reimmunized against canine distemper and hepatitis, and are identified by both neck tag and ta-

too. During the 15-day period after arrival, each animal undergoes 2 additional examinations for microfilariae. Fecal samples from each animal are examined for ova and parasites. Anthelminthics are administered as needed. Animals are observed daily for signs of illness, and treatments are prescribed daily by a veterinarian.

Dogs are housed in groups of 2 to 4 per run unless their illness or temperament requires individual housing. Dry dog food is offered free choice in tube-type feeders. Supplemental canned food is offered to sick animals to encourage food intake. Water is available from automatic, gravity operated waterers. Runs are washed daily with a detergent containing quaternary ammonia compounds applied with proportionater dispensers.

Random Source Cat Quarantine and Conditioning

Cats are purchased in an unconditioned state and undergo a 45-day quarantine in NIH facilities. At entry, each cat is examined by a veterinarian for signs of illness and immunized against feline panleukopenia and rabies. Sick and undesirable animals are rejected. Accepted cats are identified by a plastic neck band and by tatoo.

During the first 30 days, they are held in stainless steel cages, 2 to 4 cats per cage. The cages are cleaned daily with a detergent containing a quaternary ammonia compound applied with proportionater dispensers. During the second week, composite fecal samples are taken from each cage and examined for parasite ova. All cats in cages where parasite ova are found are treated with appropriate anthelminthics.

A hand-mixed diet containing moistened dry cat food mixed with several types of canned food is offered once daily. Diluted condensed milk is offered free choice as the only source of liquid during the first 30 days of quarantine. Cats are observed daily for signs of illness and are treated as prescribed by a veterinarian.

After 30 days, the cats are moved into a kennel area and held in groups of 10 or less per kennel. Resting boards, litter pans and automatic waterers are available in the kennels. Feeding procedures are continued as previously described.

Dog Morbidity and Mortality

The purchase of 30-day conditioned dogs has shifted the period of high mortality and morbidity to the vendor's facilities, resulting in a marked reduction in the illness and death of dogs within the NIH colony. Of 2,242 dogs received since January 1, 1970, 252 (11.2%) became ill and 17 (0.76%) died. Two died from fight wounds, one from enteritis, and one from general debilitation; the remaining 13 died from respiratory diseases.

The foregoing data are not representative of the problems encountered when conditioning random source dogs. To demonstrate this, monthly morbidity and mortality from all causes observed among 2,160 dogs conditioned during 1969 are shown in Figure 2. The annual morbidity averaged 54.4 percent and mortality 12.8 percent. Both rates increased slightly during midsummer and midwinter months, but there are no marked cyclic changes over the 12-month period. Figure 3 shows the same data by week of quarantine. The morbidity peaks at 23.6 percent during the second week; mortality lags slightly and peaks at 2.7 percent during the third week. The slight increase in mortality after 6 weeks probably reflects a hold over of chronically ill animals after the usual quarantine and conditioning period.

Respiratory disease causes an estimated 95 percent of all illness and death seen among random source dogs. Clinically, the syndrome be-

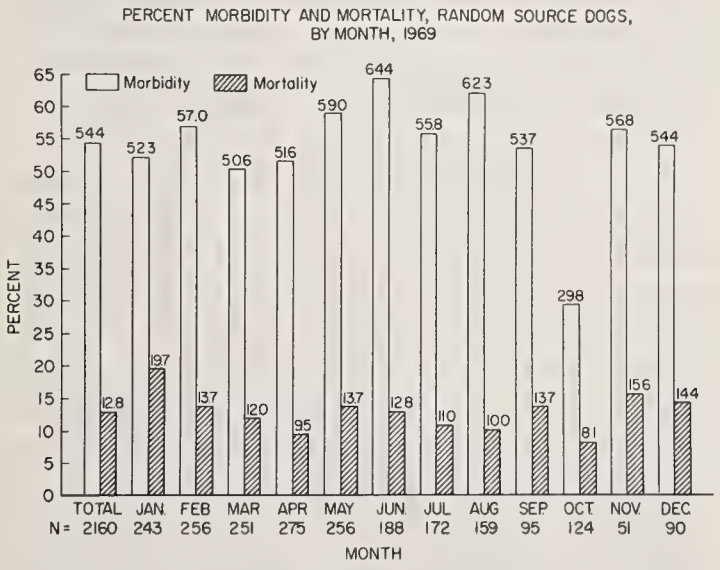


FIGURE 2.

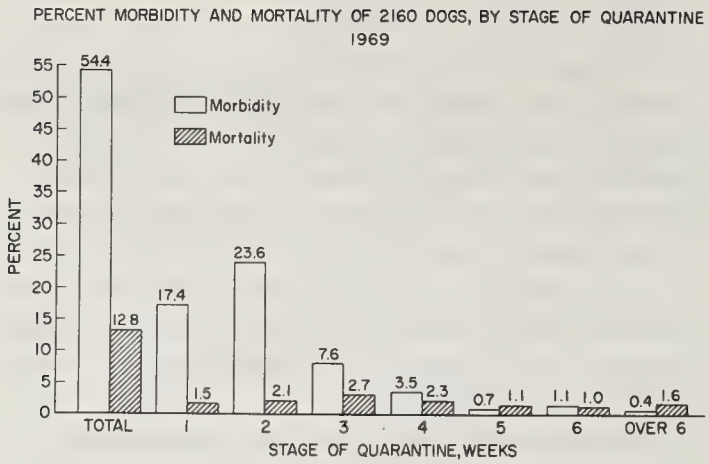


FIGURE 3.

gins with a serous nasal discharge with or without sneezing and coughing. The discharge becomes purulent within 24 to 48 hours and coughing becomes more severe. The course of the condition is not highly predictable; animals may rapidly develop severe pulmonary involvement and die suddenly from massive hemorrhagic pneumonia or may progress slowly to chronic pneumonia and either die or recover after a prolonged convalescence. Response to medication is inconsistent. Antibiotics are beneficial in some cases. A variety of antibiotics, parenteral vitamins, fluids, and forced feeding are used with variable results. Whole blood transfusions have proven beneficial in some chronic cases.

The etiology of respiratory diseases in random source dogs is not easily classified. Dogs are exposed to a variety of agents between the time that they are acquired by the animal dealer and their delivery to the research laboratory. The combination of factors, including the exposure to pathogens, stress, and the immunological status of individual dogs is believed to be related to the incidence and severity of the diseases seen during the conditioning period. For example, in 1969, pathological and microbiological examinations were made on tissues from 30 random source dogs which were killed at various stages of clinical illness. Gross lesions consisting of congestion, hemorrhage, and red and grey hepitization were observed in the lungs of 15 dogs. The following organisms were isolated from the group: *Streptococcus zooepidemicus* (11 dogs), *Bordetella bronchiosepticus* (6 dogs), *Klebsiella spp* (4 dogs), *Staphylococcus sp* (2 dogs), *Pseudomonas aeruginosa* (2 dogs), and alpha hemolytic streptococtus (1 dog). Two or more species of bacteria were isolated from the lungs of each of 4 dogs and isolations were made from 8 lungs which appeared grossly normal.

While several of the organisms listed above have been isolated by others from the lungs of dogs which died from bronchopneumonia, their roles were considered secondary to predisposing factors, such as primary viral disease, malnutrition, and exposure.[6] This is believed to be the case with quarantined random source dogs. Canine distemper virus (CDV), infectious canine hepatitis virus (ICHV), and canine herpesvirus have been isolated from the tissues of unconditioned random source dogs.[7] Isolates from 25 dogs, examined immediately after collection included ICHV (2 dogs) and CDV (10 dogs). ICHV was isolated from 7 and herpesvirus from 1 of 30 ill dogs examined after 1 to 6 weeks of quarantine. CDV and ICHV were isolated from both unimmunized dogs and dogs which had received modified live distemper-hepatitis vaccine 1 to 6 weeks prior to culturing. These and other viral agents probably play a role in the incidence of respiratory disease among random source dogs. However, canine distemper and infectious canine hepatitis are rarely recognized as clinical diseases within the quarantine colony.

Enteric parasites are not recognized as a primary cause of clinical disease in the NIH dog quarantine colony. More than 75 percent of unconditioned random source dogs accepted for quarantine harbor one or more species of hookworms, roundworms, whipworms or tapeworms. Prior to January 1, 1970, all dogs were routinely treated with dichlorvos. Currently, anthelminthics are prescribed on an individual basis for animals shedding parasite ova.

Coccidia occasionally cause enteritis in adult dogs, but the incidence of clinical disease in the quarantine colony is low when compared with the prevalence of oocyst shedding. The organisms are apparently wide-spread in the colony despite the level of sanitation maintained. A study of 155 dogs in 1969 revealed that 12.9 percent of the dogs were shedding oocysts at

entry, while 65.6 percent of 154 were shedding 28 days later. There is no attempt to eliminate coccidia except when diarrhea is accompanied by oocyst shedding.

Since the majority of the dogs quarantined are used in cardiovascular research, the elimination of microfilaria-infected dogs is of major concern in the quarantine program. A single examination, using both direct microscopic examination and the modified Knott's[8] test, has proven inadequate to identify all of the infected dogs. No attempt is made to distinguish microfilaria of *Dirofilaria immitis* from *Dipetalonema spp;* all dogs with microfilariae are rejected. Results from single and multiple examinations for microfilaria are shown in Table I. Multiple examinations increase the incidence of microfilaria detection. Single examinations revealed microfilaria in 4.1 percent of the dogs while 3 examinations revealed microfilaria in 7.6 percent of the dogs. To reduce the number of microfilaria positive animals received from the vendor, the laboratory examines dogs for microfilaria while they are held in the vendor's facility. Blood is collected, placed in the Knott's solution, and shipped to the laboratory for evaluation. The intent of the program is to examine each dog at least 3 times prior to its delivery to NIH. Of 2,756 animals examined under this program, 1,757 received 2 examinations and 41 were examined 3 times. However, the NIH has diagnosed microfilariasis in an additional 5.8 percent of the 1,377 dogs delivered since the initiation of this procedure.

Various disease conditions exist with low incidence among quarantined random source dogs. Those frequently seen include dermatitis (including parasitic, fungal, bacterial and idiopathic), otitis, opthalmic problems, fight wounds, metabolic diseases and, rarely, neurological diseases. These are insignificant in comparison to the respiratory disease problems since they rarely cause mortality.

TABLE I.—*Results of Microfilaria Screening of Random Source Dogs*

Category	Number tested	Positive No./Percent			
		Test 1	Test 2	Test 3	Total
1 Test	2,750	113/4.1			113/4.1
3 Tests (weekly)	895	23/2.6	36/4.0	9/1.0	68/7.6
At Vendor					
1 Test	2,756	212/7.7			212/7.7
2 Tests	1,757		103/5.9		103/5.9
3 Tests	41			3/6.8	3/6.8
After Testing					
At Vendor					
3 Tests	1,377	41/3.0	17/1.3	20/1.5	78/5.8
DIRECT SMEAR	1,377	35/2.5			
MODIFIED KNOTTS	1,377	41/3.0			

Cat Morbidity and Mortality

Cats are purchased unconditioned and are held for a 45-day quarantine period in the NIH facilities. During fiscal year 1971, 35.1 percent of 2,510 cats became ill and 14 percent died. Figure 4 presents the monthly rates for morbidity and mortality from all causes during this period. Morbidity was highest during the winter and lowest during spring and autumn months. Mortality rates were variable, with November and July being peak months. The same data are shown by week of quarantine in Figure 5. More than half of the morbidity and mortality occurs during the first week; 80 percent during the first 3 weeks. A large number of early deaths are caused by feline panleukopenia, while illness and death occurring later in the quarantine is often caused by respiratory or enteric disease.

Respiratory disease is the major cause of morbidity among random source cats. The course of the disease syndrome is usually similar in the early stages to the condition seen in dogs; however, the mortality rate is lower. Several viruses have been identified as causes of fe-

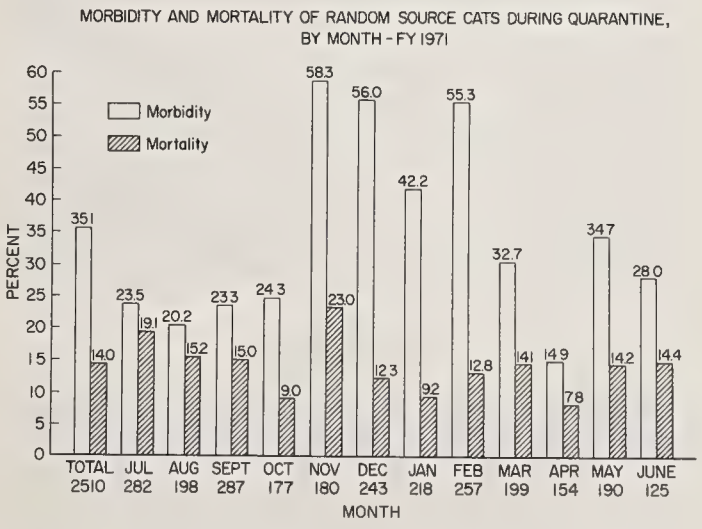


FIGURE 4.

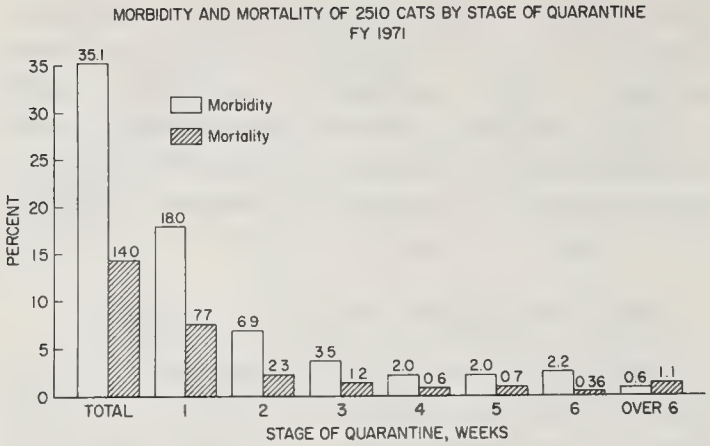


FIGURE 5.

line respiratory disease, the most common being feline pneumonitis and feline rhinotracheitis viruses.[9] Numerous others have been isolated from the respiratory tracts of cats, but their roles in disease and their relationship to other isolates are not yet fully understood.[10] There are undoubtedly many viral, mycoplasmal, and bacterial agents involved in feline respiratory disease as seen in the quarantine colony. Most cases respond favorably to wide spectrum antibiotics; however, some become chronic or progress to pneumonia with subsequent death. Ocular lesions compatible with feline rhinotracheitis virus infection are frequently seen.[11]

Endoparasites are found frequently, but cause no obvious clinical signs. About 75 percent of fecal flotation examinations are positive for hookworms, and 45 percent for roundworms. Fifty-five percent of the cats are found to harbor tapeworms. Cats are treated with anthelminthics, based on results of fecal examinations during the first week of quarantine.

Other disease conditions are recognized in low incidence among random source cats. They include, but are not limited to, dermatophytic fungal infections, infectious feline peritonitis, abscesses, oral and corneal ulcers, and enteritis.

SUMMARY

Morbidity and mortality rates among random source dogs and cats are high during their 45-day quarantine and conditioning period. The most serious problem among random source dogs is respiratory disease which is the principal cause of mortality. Cats suffer their greatest mortality early in quarantine, caused primarily by feline panleukopenia. Respiratory disease is also of major importance and causes considerable morbidity and mortality in cats. Most infectious diseases are attributable to the exposure of the animals to a wide variety of pathogens while they are stressed during holding and transport by pounds and vendors before and during delivery to the research institutions.

REFERENCES

1. *Institute of Laboratory Animal Resources News*, National Research Council-National Academy of Sciences Vol. XII, No. 1: iv, 1968.
2. *Institute of Laboratory Animal Resources News*, National Research Council-National Academy of Sciences Vol. XIII, No. 1: iv, 1969.
3. *Institute of Laboratory Animal Resources News*, National Research Council-National Academy of Sciences Vol. XIV, No. 1: vi, 1970.
4. ZINN, R. O. The Research Dog. J.A.V.M.A. 153, No. 12, 1883–1886, 1968.
5. POTKAY, S., and BACHER, J. The Research Dog: Random Source or Colony Reared, The Proceedings of The National Conference on Research Animals In Medicine, NHLI, pp. , 1972.
6. ARCHIBALD, J. The Lungs. In *Canine Medicine*, 2nd ed., P. H. HOSKINS, et al., editors, America Veterinary Publications, Santa Barbara, Calif., pp. 171–172, 1964.
7. SMITH, K. O. et al. Isolation of Viruses from Primary Dog Cell Cultures and the Occurrence of Viral Antibody in Donor Dogs. Proc. of the Soc. for Expt. Biol. & Med. 133, 560–567, 1970.
8. KNOTT, J. A Method for Making Microfilarial Surveys on Dog Blood. Trans. of the Royal Soc. of Tropical Medicine & Hygiene XXXIII, No. 2, 191–196, 1939.
9. STUDDERT, M. J., and MARTIN, M. C. Virus Diseases of the Respiratory Tract of Cats: 1. Isolation of Feline Rhinotracheitis Virus. Australian Veterinary Jour. 46:99–104, 1970.
10. WILNER, B. I. *Classification of the Major Groups of Human and Other Animal Viruses*. 4th Edition, Burgess Publishing Co., Minniapolis, Minn., pp. 43 & 106, 1969.
11. BISTNER, S. I., SHIVELY, J. N., and SCOTT, F. W. Ocular Manifestations of Feline Herpesvirus Infection. J.A.V.M.A. 159, No. 10, 1223–1237, 1971.

DISCUSSION

CHARLES B. THAYER, University of Iowa: Why did you pick the time of forty-five days for your quarantine period?

DR. PALMER: The question was: why did we pick the time of forty-five days for quarantine? As I said, the history of our quarantine period was given by Zinn some years ago. The period started at two weeks, but in 1963, there was a rabies scare which caused some consternation and, after consideration, it was decided that thirty days was required to make the animal safe. In 1967, this period was changed to forty-five days with the intention of eventually going to sixty days. After considerable experience with the forty-five day quarantine, it was decided that more than forty-five days would not add considerably to the conditioning of the animal or improve his status. That was about all you could do. We have found that the forty-five day conditioned dog is much superior to the thirty-day conditioned dog. The animal has had a chance to resolve many of the pneumonic lesions and is a much better animal.

DR. THAYER: One more question, please. If you're holding them for forty-five days because of a rabies scare, you're kidding yourself, of course, because the incubation time for rabies is after six months.

DR. PALMER: No, we're not holding them forty-five days for a rabies scare.

DR. THAYER: Your figures show that at least 8% of your cats' mortality occurred in the first three weeks and over 9% of your dogs' mortality occurred in the first four weeks. I see no reason for keeping the rest of them for a longer period of time.

Do you differentiate between those dogs and cats which are going to be used for an acute project and those which are going to be used for a chronic investigation, or, is everything held this length of time?

DR. PALMER: We have no way of determining what the animal is going to be used for when it leaves our facility.

DR. THAYER: That's where our tax money goes.

DR. PALMER: So we should use sick cats for acute experiments? Sick cats or dogs? But how do you know a cat or a dog is healthy after fifteen days or even thirty days?

DR. THAYER: If you can't screen them with the tests you have and clinical observation, then I think you need a new veterinarian.

DR. PALMER: You don't agree that we need more prolonged conditioning?

DR. THAYER: Certainly not for acute studies.

DR. PALMER: Everyone's entitled to his opinion.

ESTELLE H. GELLER, Albert Einstein College of Medicine: I have two questions. One, what happens to the dogs that you reject because of microfilaria? Aren't they sold to someone else?

DR. PALMER: Yes ma'am. They are sent back to the vendor and we have no control over what he does with them.

DR. GELLER: The second question is: have you looked for oocysts or found oocysts in the cat species? I'm asking that particularly because of the toxoplasma scare that you see in the newspapers.

DR. PALMER: We use primarily a zinc sulfate flotation technique. We also do a direct examination in saline; a direct wet preparation.

DR. GELLER: And, in this testing you haven't seen oocysts?

DR. PALMER: We have not tried to identify the oocysts as those of toxoplasma.

DR. GELLER: But, have you seen many oocysts in general? I mean, regardless of identifying this species?

DR. PALMER: Yes.

DR. GELLER: Do you have the incidence of the oocysts in cats? You gave the percentages of the other ones.

DR. PALMER: No, I do not have the incidence in cats.

GUILLERMO PACHECO, NIH, Bethesda, Md.: May I just comment briefly on the oocysts in cats? I think it's been very clearly established that toxoplasma oocysts are shed for a very limited period of time. And there's no evidence at all to indicate that the isospora or immitis which are found in cats are the least bit transmissible to other animals.

DR. GELLER: I'm with you on that, but you see articles in "Newsweek" and "The New York Times," and "McCall's" and all these places scaring everybody.

GLEN G. WRIGLEY, Buckshire Corp.: Have you used dichlorvous as a helminthic in your felines?

DR. PALMER: We have used it. In fact, we used it routinely for a short period of time. We

were concerned about the fact that it was not cleared for cats. It's not available now on the market, so we have not been able to use it in any of our animals. We are now routinely using Vermiplex and Yomesan.

STEVAN SIBINOVIC, Bionetics Research Laboratory, Kensington, Maryland: I congratulate you on the number of animals which you observed and I think it is excellent. I'd be interested in the incidence of spontaneous neoplasm in the dogs and cats?

DR. PALMER: We rarely see neoplasms, extremely rarely: but, they're only in our facility for a period of forty-five days. These are relatively young animals usually and they don't stay very long.

DR. SIBINOVIC: Thank you.

CHAIRMAN: What is the incidence of pneumonia or other critical entities in dogs held after several weeks at the quarantine area?

DR. PALMER: Pneumonia can occur at any time. We suffered an epizootic during 1969 of a clinical syndrome, which we called the bleeder syndrome, where an animal appeared healthy one day and was dead the next with a hemorrhagic type of pneumonia. This disease occurs virtually at any time. In the animals which we're purchasing now, after thirty days of quarantine the incidence of pneumonia has been 13 out of 17 deaths, or 13 of 2,242 animals. I've not figured out the percentages. I don't have good data to show accurate incidences of these diseases. For instance, dermatophytic fungus, ringworm, is a very common disease in cats, but it's not a major problem in our programs because the animals can still be used for certain things. The greatest number of pneumonias in dogs occurs during the first thirty days. And yet, in many of the dogs at the end of the thirty day period, if you examine carefully, you can detect consolidated lungs, which are not normal at that point. They need additional time to develop, yet few of those animals die.

CHAIRMAN: I asked that because my area handles most of the dogs produced by the NIH quarantine and we noticed the difference in the thirty day quarantine dog and the forty-five day quarantine dog in the laboratory. And we have emphasized this procedure. We not only quarantine dogs, but we've transported them from the Animal Center to the NIH reservation which seems to be a tremendous stress. The dogs will break with pneumonia or other conditions the day after arrival. Most of them stay around the laboratory pre-op holding area for several days before being used in the laboratory. So we have found an advantage in this.

KEITH G. MCCULLAGH, Cleveland Clinic: Can I just ask you who your present vendor is and how you made the decision on which company to choose?

DR. PALMER: We purchase on an annual contract. The current vendor is Lone Trails Kennels in Friedensberg, Pennsylvania. The contract is negotiated. We have, in the past, had two vendors to supply our needs. The use of random source dogs at NIH has dropped from almost 5,000 in 1966 and 1967 to about 2,000 or less at the present time. Dr. Potkay will tell you all about this Sunday morning. We don't really select the vendor, although we try to buy from the best available source and we require that the man pass the inspection by NIH personnel; not just that he's a licensed vendor.

DR. THAYER: I'm sorry, but I can't stay out of this. You, Dr. Pierce, mentioned that you saw a great difference in your NIH dogs which were quarantined thirty days and forty-five, because they were transported to your facility. I don't know how far your facility is from where the quarantine area is. Every one of our dogs, after its quarantine period, is transferred; admittedly it's only a five mile transfer, but he is transferred. The point that I'm getting at is that I still think there's something wrong. When I look at your mortality and morbidity figures and, in particular, the testing for the heart worm business about which you were talking, I say you're still not doing the program you should. Mine are less than yours and I don't do anything like you do, you see? That's my only comment.

Furthermore, I feel that you have made the same mistake that a number of other institutions have been making in not having two classes of dogs and cats, whether they be separate quarantine colonies or whatever—but one design for the acute animal. These animals should certainly have some screening and they should have some quarantining, but they do not

need what the chronic animal needs. And these two classifications should be separate and the price of these animals should be separate, and the length of quarantine can be separate. The reason I brought up the bit about rabies before was because you said you went to a thirty day quarantine because you had a rabies scare. And I couldn't quite understand the logic of that.

DR. PALMER: I think I can answer that. I won't assume the responsibility for having changed it. The fifteen day period at that point was just not considered long enough and the impetus came from the rabies scare, but I think it would have been changed anyway. And I don't agree with you that we shou'd issue acute animals because I don't think that any quarantine less than forty-five days is good enough. Very honestly, we would love to be out of the business of supplying random source dogs. We would like to supply colony-reared dogs and we hope that eventually we'll get there.

DR. THAYER: Why not go to six months?

DR. PALMER: Because we don't feel that anything beyond forty-five days will improve the quality of the animal.

GEORGE H. BARNEY, Vanderbilt University: Do you believe that the incidence of respiratory diseases in dogs would be markedly decreased if you selected them at the dog pound instead of purchasing them from a vendor?

DR. PALMER: No, I think the only way you could reduce it would be to hold the animals in a more isolated state from the time the dog catcher picks them up and to immunize them against those common viral diseases for which immunization procedures exist. Now, I don't know of anyone who's tried this. Perhaps someone in the audience has, but I think that, in the monkey, for instance, it's been found that if the animals are kept in isolation from the trap to the laboratory, they could produce animals which were much more healthy.

DR. BARNEY: This might interest you. For years we kept all our dogs in one central building. Then, in 1969, we constructed an animal research facility in the country. We conditioned our dogs there for a week and then vaccinated them for distemper and hepatitis. The instance of this upper respiratory infection has practically disappeared. We like this procedure and it works well for us.

DR. PALMER: You immunize as soon as they come in?

DR. BARNEY: Yes.

DR. PALMER: And what is your source? Is it a vendor or a pound?

DR. BARNEY: We select these dogs at the dog pound. For instance, if they have fifty dogs, we'll select perhaps five or six, just by general health, skin condition, presence of diarrhea or respiratory signs. This has worked well for us.

DR. PALMER: You could probably do a lot better if you went out and picked them up off the street before the dog pound got them.

CHAIRMAN: I'd like to make two comments. One, the transportation from the Animal Center to the NIH reservation which I mentioned involves a distance of thirty-five miles or usually an hour on a truck and it is a stress, and the dogs do break. Another thing, in line with Dr. Pacheco's and Dr. Palmer's ideas, dogs, especially random source dogs used in the laboratory without examination of the right ventricle and pulmonary artery, can never be said to be free of heart worms. They are asymptomatic and do exist as a problem in the laboratory. There are many occasions of accidental findings of the worms: there's negative blood, but the adults are there; they're just not producing microfilaria at that stage.

CONGENITAL DEFECTS OF THE CARDIOVASCULAR SYSTEM IN DOGS

D. F. Patterson*

The development of clinical cardiology in veterinary medicine has resulted in the recognition and study of a wide variety of congenital cardiovascular defects in dogs, many of which are close counterparts of human disorders. These include the more common anatomic forms of congenital heart disease and a number of congenital abnormalities in cardiac excitation recognized by their electrocardiographic features. This paper illustrates how studies of some of these animal models have led to new insights into etiologic and pathogenetic mechanisms.

Epidemiologic and genetic studies have shown that the common anatomic defects of the cardiovascular system in dogs have a genetic cause. The underlying genetic abnormality is complex but lesion-specific in nature. Breeding experiments have proven the feasibility of producing dogs with specific defects for a variety of research purposes by selective breeding. The genetic features, sequelae, and pathogenetic mechanisms in hereditary canine patent ductus arteriosus are reviewed.

A number of forms of naturally occurring congenital heart disease have been utilized in studies of cardiac pathophysiology not possible in man. Contributions of this work include the identification of a genetically determined variant in right ventricular wall thickness without other defects as a cause of the incomplete right bundle branch block pattern, and the first anatomic and electrophysiologic delineation of an accessory atrioventricular conduction pathway in the Wolff-Parkinson-White syndrome. Pathologic studies suggest that congenital defects of the atrioventricular conduction system may exist in dogs, pointing to another potential model of human cardiovascular disease. Other examples of the use of dogs with congenital cardiovascular defects for physiologic studies include investigations of the spread of activation in right ventricular hypertrophy associated with various forms of congenital heart disease, and chronic studies of coronary blood flow in dogs with fibrous subaortic stenosis.

INTRODUCTION**

During the past 20 years, the development of clinical cardiology in veterinary medicine has resulted in the recognition and study of a wide variety of congenital cardiovascular defects in dogs. These include a large group of anatomic malformations of the heart and major vessels and a number of congenital disturbances in cardiac excitation which are recognized by their electrocardiographic features. Many of these defects bear a close resemblance to cardiovascular abnormalities found in man, in their frequency of occurrence, their anatomic details, and in the physiologic derangements they produce. It is the purpose of this paper to show how studies of some of these animal models have led to new insights into etiologic and pathogenetic mechanisms. For a more detailed discussion of the individual defects, the reader is referred to previous reports.[1-12]

GROSS ANATOMIC DEFECTS OF THE CARDIOVASCULAR SYSTEM IN DOGS

Gross malformations of the heart and great vessels have been shown to occur in 0.5 to 1% of dogs presented to a veterinary clinic.[1-2] Twenty-five different lesions have been reported,[4] including the most common forms of congenital heart disease found in man. There appear to be some similarities in the distribution of the various types of anatomic defects in man and in the dog. A comparison of the relative frequencies of the common malformations in two veterinary clinic population studies and the mean of percentages from heart clinics in three children's hospitals[13] is shown in Table I. (The more desirable comparison of the incidence of defects at birth cannot be made because of the lack of reliable data in the dog.)

While there are differences in the relative frequency of individual defects, it is noteworthy that 6 lesions comprise over 60% of all defects in the dog and human series: Patent ductus arteriosus, pulmonic stenosis, aortic stenosis, ven-

* University of Pennsylvania School of Veterinary Medicine.

** Supported by U.S. Public Health Service Grants HL 10995 and 4885 from the National Heart and Lung Institute, National Institutes of Health.

TABLE I.—*Comparison of the Relative Frequencies of Various Types of Cardiovascular Malformations in Human and Veterinary Clinics*

	Percent of all Heart Defects		
	Dog[14]	Dog[3]	Human[13]
Malformation	Veterinary Clinics–NCI Survey (N=303)	U. of P. Veterinary Clinic (N=289)	Children's Clinics (N=8674)
Patent Ductus Artreiousus	31.0	25.3	14.9
Pulmonic Stenosis	13.5	17.6	10.5
Aortic Stenosis	3.0	12.3	5.6
Ventricular Septal Defect	9.2	6.2	24.2
Atrial Septal Defect	3.0	3.7	12.4
Tetralogy of Fallot	*	3.4	10.9
Subtotal	59.7	68.5	78.5
Persistent Right Aortic Arch	10.9	7.1	*
Coarctation of Aorta	0.0	0.0	4.9
Other	29.4	24.4	16.6
Total	100.0	100.0	100.0

* Frequency not given—included in "Other."

tricular septal defect, atrial septal defect, and tetralogy of Fallot. Ventricular septal defect was the most commonly diagnosed lesion in the human series, followed by patent ductus arteriosus. In both dog studies, patent ductus arteriosus was the most commonly diagnosed defect, while pulmonic stenosis was the next most common. There are two obvious differences between dog and man in their susceptibilities to particular anatomic defects. Coarctation of the aorta is only slightly less common than the 6 most frequently reported malformations in man, but it was not observed in either veterinary clinic population survey. A recent report constitutes the only documented occurrence of aortic coarctation in a dog.[12] Persistent right aortic arch as an isolated malformation is found much more frequently in the dog than in man as is indicated by the surveys in Table I and a review of the veterinary literature.[4]

ETIOLOGY OF CARDIOVASCULAR MALFORMATIONS IN THE DOG

Studies of the breed distribution of cardiovascular malformations in dogs presented to the University of Pennsylvania Veterinary Clinic suggested that purebred dogs are more susceptible to these birth defects than are dogs of mixed breeding, the prevalence rate being approximately three times greater in purebreds than in mongrels.[2,3] This finding has been confirmed by Mulvihill in an independent survey of 10 other university veterinary clinics participating in a Veterinary Medical Data Program conducted by the National Cancer Institute.[14] In both the University of Pennsylvania and National Cancer Institute surveys, there was a non random distribution of anatomic defects among the breeds, suggesting that certain breeds are more susceptible to some defects than others.[3,14] Breed predispositions suggested by the two veterinary clinic population surveys and a review of the world literature[4] are given in Table II.

The apparent predisposition of certain breeds to specific anatomic defects is consistent with the hypothesis that the underlying abnormalities in development are caused at least in part by lesion-specific genetic determinants concentrated in these breeds.[3,4] The higher prevalence rate of congenital heart defects in purebred than mixed dogs further suggests that these genetic determinants may be single genes or polygenic sets that behave as recessives.

Tests of the hypothesis of lesion-specific genetic determinants have been conducted in a breeding colony of dogs with congenital heart disease.[3,5,6,7] Affected animals were obtained for the colony through the cooperation of owners, kennels, and veterinarians. Young animals were raised to maturity and used in test matings. Initial tests involved the mating of affected pairs, matched for lesion and breed according to the suspected breed predispositions (Table II). In this way, the highest probability

TABLE II.—*Breed Predispositions to Congenital Heart Defects Suggested by Epidemiologic Data and Literature Review*

Defect	Breed	Source
Patent ductus arteriousus	Poodle	3, 14
	Collie	3
	Pomeranian	3
	Cocker Spaniel	14
Pulmonic stenosis	English Bulldog	3
	Fox Terrier	3
	Chihuahua	3
	Beagle	3
Aortic stenosis	Boxer	3, 4, 14
	German Shepherd	3
	Newfoundland	3
Ventricular septal defect	Siberian Husky	14
Persistent right aortic arch	German Shepherd	3, 4, 14
	Irish Setter	3
Tetralogy of Fallot	Keeshond	3

of producing affected offspring was achieved, regardless of the mode of inheritance which might be operating. The lesions tested were patent ductus arteriosus in Poodles, pulmonic stenosis in Beagles, subaortic stenosis in Newfoundlands, persistent right aortic arch in German Shepherds, and tetralogy of Fallot in Keeshonden. In each instance, hereditary transmission of congenital heart disease was demonstrated, the incidence of defective offspring reaching as high as 90% in some matings.[4,6] The cardiovascular defect in the affected offspring was usually the same or closely related to that in the parents. These findings confirm the hereditary, lesion-specific nature of the defects tested, and indicate that through selective breeding a high incidence of offspring with predictable cardiovascular defects can be produced for a variety of research purposes.

Further genetic studies have shown that although some of the lesions tested are transmitted in a manner superficially resembling autosomal dominant inheritance, none can be explained on a simple genetic basis.[6,7] The most completely analyzed defect is patent ductus arteriosus. Further discussion of gross anatomic defects will be confined to this anomaly.

HEREDITARY PATENT DUCTUS ARTERIOSUS (PDA) IN POODLES

Defective closure of the ductus arteriosus in Poodle dogs is a localized developmental anomaly determined by multiple genetic factors which appear to act additively.[5] Thus, what appears on the surface to be a discontinuous trait actually results from a more or less continuous underlying distribution of genetic determinants which affect the normal ductal closure mechanism. Discontinuity at the gross phenotypic level occurs when their cumulative effects exceed some critical threshold and the ductus arteriosus fails to close. Careful scrutiny of offspring from matings of dogs with PDA shows that there are not simply two phenotypic classes. In addition to normal pups, and those with PDA (Figure 1), there is an intermediate phenotype in which the lumen of the ductus arteriosus is obliterated at the pulmonary arterial end, while a variable portion of the segment adjoining the aorta remains open. This gives rise to a funnel-shaped *ductus diverticulum* which appears as an outpocketing of the ventral (sternal) wall of the aorta at the site of the ostium of the ductus arteriosus (Figure 2, bottom). This *forme fruste* of hereditary PDA is clinically silent, being detected in living animals only through the use of aortic angiography (Figure 2, top). In test matings, dogs with ductus diverticulum did not differ markedly from dogs with fully patent ductus arteriosus in the ability to transmit defective ductal closure to their offspring.[5]

The results of experimental matings of dogs with PDA are consistent with a polygenic model having two developmental thresholds.[5] The first threshold separates complete closure of the ductus arteriosus from ductus diverticulum, and the second separates ductus diverticulum from complete patency of the ductus arteriosus. These two thresholds may be looked upon as two distinguishable points along a continuous scale of "liability" to abnormal development which extends in both directions. Assuming that liability is continuous and normally distributed, the mean and standard deviation of liability of a group of individuals may be determined from the incidence of the three phenotypic classes (normal, ductus diverticulum, PDA) by referring to a table of the normal curve.[5] Figure 3 shows the distribution of liability to defective ductal closure in three groups of offspring, each receiving a different proportion of its genome from dogs with PDA. As the PDA-derived portion of the genome (r value in Figure 3) increases from ½ to ¾ to 1, the mean liability of the offspring is progressively shifted to the right, beyond the thresholds for ductus diverticulum and PDA.

Left Heart Failure and Pulmonary Hypertension in Pups with Hereditary PDA

While increasing phenotypic severity beyond the threshold for PDA is not considered in the model shown in Figure 3, other evidence suggests that with an increasing "dose" of genes predisposing to abnormal ductal closure, there is a continuing increase in the severity as well as the incidence of the underlying lesion. The

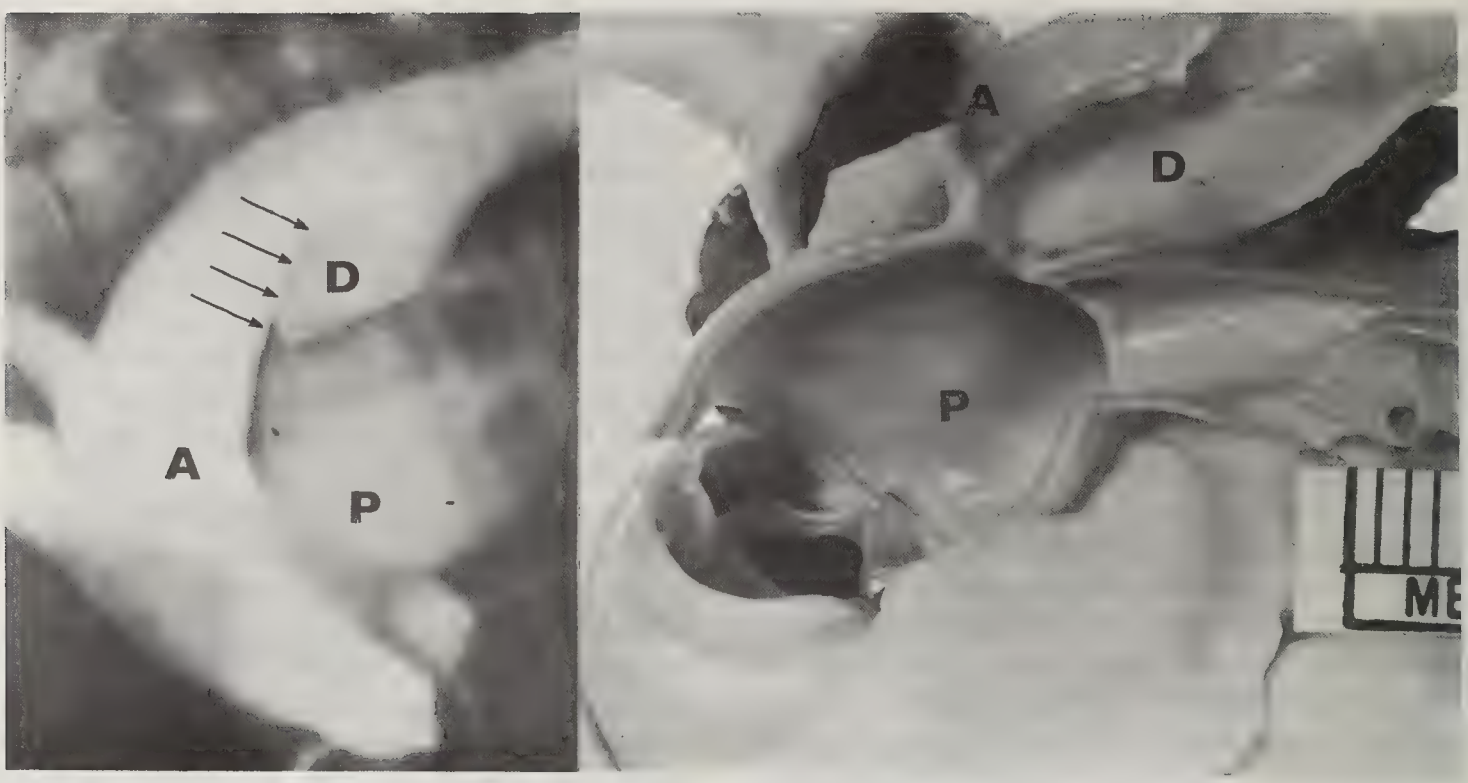


FIGURE 1.—Patent ductus arteriosus with left-to-right shunt. Left: Left lateral aortic angiogram of a 12-week-old male mixed Poodle with a large funnel-shaped PDA and left-to-right shunt. After injection of contrast medium into the aorta (A), the ductus arteriosus (D) and dilated pulmonary artery (P) were visualized. The opposed walls of the ductus arteriosus and aorta are seen as a radiolucent line (arrows). Right: Left lateral view of the heart of 16-week-old female Poodle with a large funnel-shaped PDA (D); a left-to-right shunt was present in life.

Reprinted from reference #5 by permission of the American Heart Association, Inc.

size of the ductal lumen in pups with patent ductus arteriosus varied from a small channel of little hemodynamic significance to a large vessel approaching the diameter of the ascending aorta. In pups which had large ductal lumens, there was a high incidence of left heart failure and a smaller incidence of severe pulmonary hypertension with right to left or bidirectional shunts. The incidence of these sequelae to PDA rose from 33% in pups receiving 50% or less of their genomes from dogs with PDA, to 78% in pups whose parents both had PDA.[5] This finding is consistent with the view that the genes predisposing to PDA have a dose related effect on the eventual size of the lumen in ductuses which fail to close.

Observations on the natural history of left heart failure and pulmonary hypertension in pups with hereditary PDA have led to some interesting evidence regarding the pathogenesis of these two sequelae, evidence which may aid in understanding the factors influencing these serious complications of congenital heart disease in young children. Daily examinations of the offspring of dogs with PDA have shown that PDA can be detected in some individuals as early as the second day after birth and that most affected pups are recognizable by the end of the second week.[7] A high incidence of left heart failure occurs between the second and fifth week, preceeded by auscultatory, radiographic, and electrocardiographic signs of an increasing left to right shunt with left ventricular overloading. Pups with the syndrome of left heart failure invariably die with severe pulmonary edema before the end of the fifth week unless surgical ligation of the ductus arteriosus is carried out. If signs of left heart failure did not become clinically evident by the fifth week, the animals usually survived to adulthood.

These observations suggest that the second through the fourth week of postnatal life is a period of critical circulatory adjustment in the dog, during which a downward changing pul-

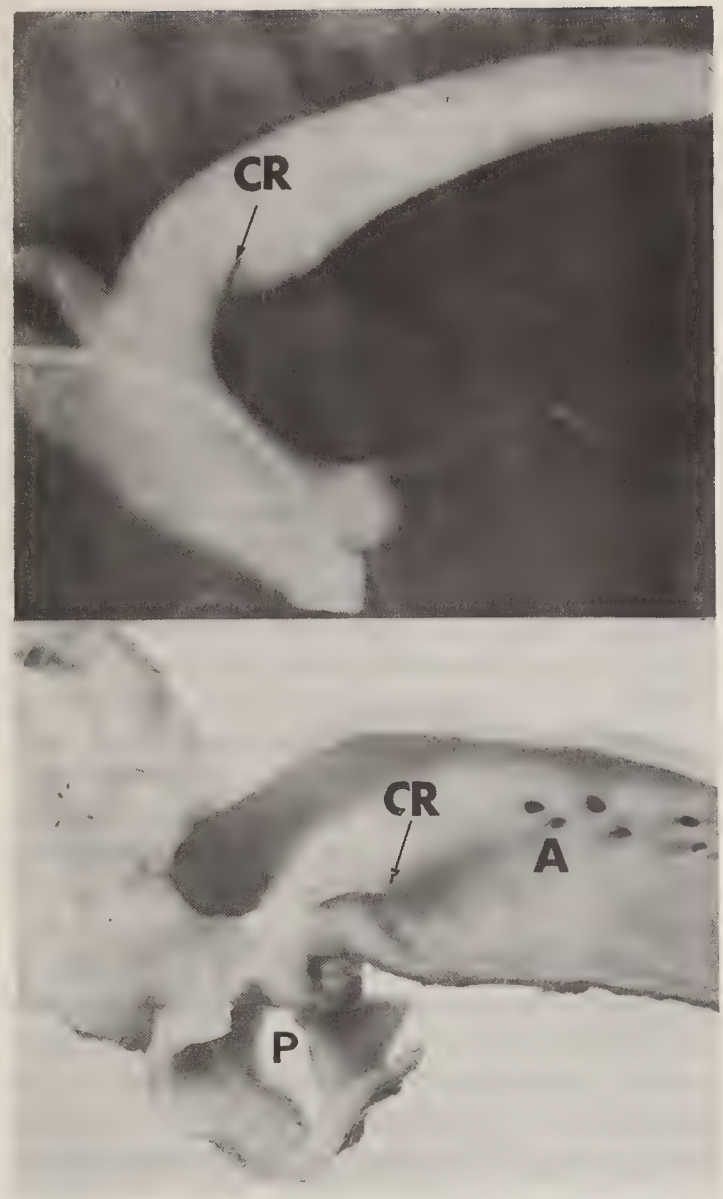


FIGURE 2.—Ductus diverticulum. *TOP:* Lateral aortic angiogram of a 13-week-old male pup from a cross between a male Poodle with a ductus diverticulum and a normal female Coonhound. A funnel-shaped ductus diverticulum can be seen over the heart base. The distal extremity of the diverticulum extends well beyond the outer border of the aortic wall. The crista reuniens (CR) is formed by the adjacent walls of the ductus arteriosus and aorta. *BOTTOM:* Sagittal section of the aorta (A), ductus diverticulum, and pulmonary artery (P) of the same dog. The ductus diverticulum extends beneath the crista reuniens (CR) to about one-half the length of the ductus arteriosus.

Reprinted from reference #5 by permission of the American Heart Association, Inc.

monary vascular resistance favors an increasing left to right shunt and volume overloading of the lungs and left heart, if the ductus arteriosus remains patent. This view is consistent with the observations of Rudolph and co-workers,[15] who have followed pulmonary arterial and right ventricular pressure changes in normal unanesthetized pups from 1 to 36 days of age. Their studies show a rapid fall in peak right ventricular pressure during the first week, followed by a more gradual decrease to adult levels over the next 4 weeks. Phillips et al.[16] found a similar fall in pulmonary arterial pressures and noted that lumen/wall ratios of the small pulmonary arteries in pups increased to adult values by four weeks of age.

Pups which developed severe pulmonary hypertension with right to left or bidirectional shunts were distinguishable from other pups with PDA by the end of the second week. There was no evidence of substantial left to right shunting at any time: continuous murmurs appeared only briefly or not at all, and despite the presence of a patent ductus arteriosus which approached the aorta in size, signs of left heart failure did not ensue. In electrocardiographic studies, the "right ventricular hypertrophy" pattern seen normally at birth did not evolve toward the adult pattern of left ventricular dominance as it did in normal pups and other pups with PDA. Cyanosis of the caudal portion of the body was visible in some animals. Cardiac catheterization at 8 to 10 weeks of age revealed pulmonary arterial pressures which equaled or exceeded aortic pressure throughout part or all of the cardiac cycle. The evolution of clinical signs suggests that in pups of this type the usual postnatal fall in pulmonary vascular resistance fails to occur. As has been discussed by Rudolph, failure of maturation of a low resistance pulmonary vasculature in such cases may be the result of constant exposure of the pulmonary arterial circulation to the high pressure of the systemic circuit.[17] Such a condition would be expected if the size of the communication between the two circulations is sufficiently large.

Pathogenesis of Hereditary PDA

Closure of the ductus arteriosus normally occurs in two stages. Active constriction of the muscular wall of the vessel takes place within hours after birth, resulting in *physiologic closure.* Contraction of ductal smooth muscle ap-

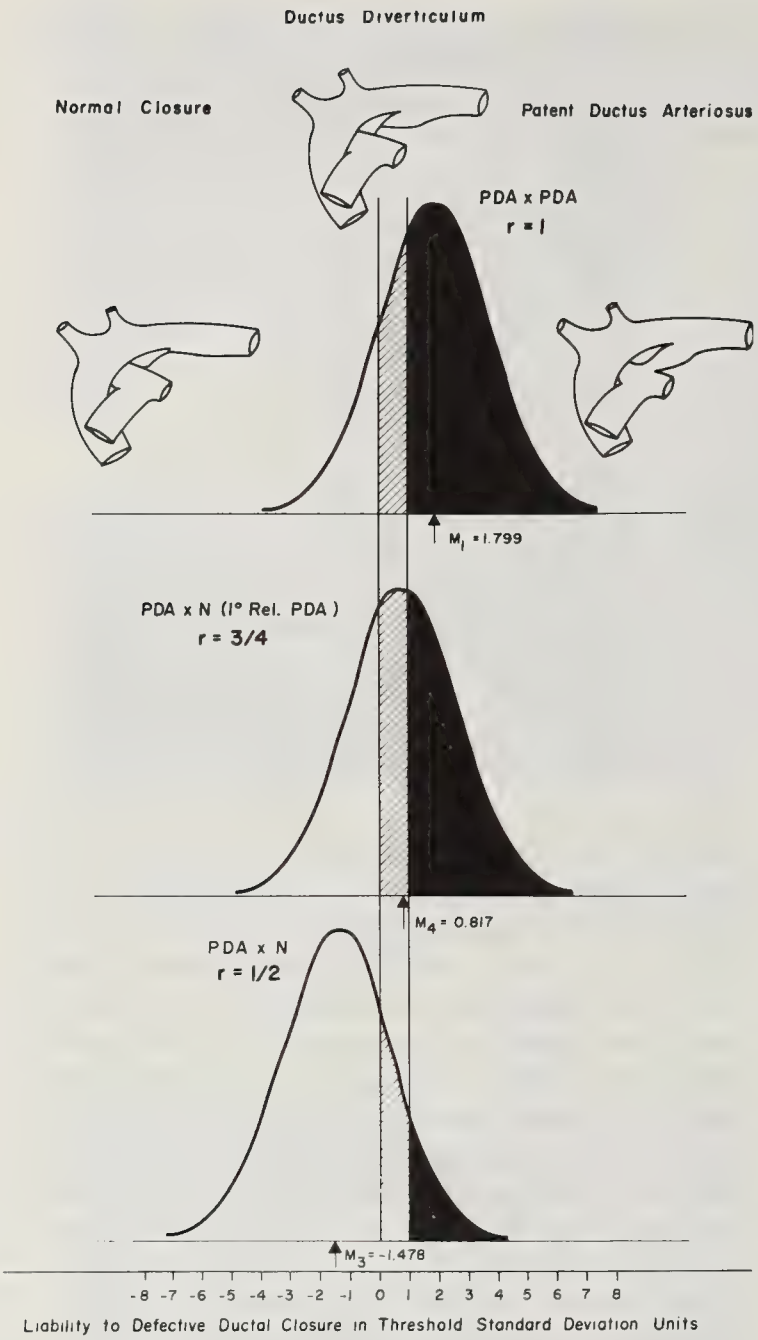


FIGURE 3.—Threshold model of hereditary patent ductus arteriosus

The distribution of liability to defective ductal closure in offspring of 3 types of matings is compared. The location of the mean liability of each group is indicated as measured from the lower threshold for defective ductal closure (threshold between normal and ductus diverticulum). The mean liability of offspring of matings between PDA dogs and normal dogs with no family history of PDA (PDA × N) lies below the lower threshold, while the mean liability of offspring of matings between 2 dogs with PDA (PDA × DPA) lies well above the threshold for PDA. The mean liability of offspring of matings between PDA dogs and the first degree relatives of dogs with PDA [PDA X N (1° Rel. PDA)] lies about half way between the other 2 means. The r values represent the proportion of genes each group of offspring has in common with dogs with PDA. Reprinted from reference #6 with permission.

pears to be mediated by the increase in blood oxygen tension which follows the onset of breathing. The response to oxygen is demonstrable both in the intact fetus[18] and in the isolated perfused ductus arteriosus.[19,20] While norepinephrine,[19] acetylcholine,[19] and bradykinin[21] have also been shown to cause constriction of ductal smooth muscle, there is no evidence that these agents play a major role in the normal physiologic closure mechanism. *Anatomic closure* of the ductus arteriosus occurs over a period of weeks after birth, through an extensive reorganization of the histologic structure of the ductal wall. The vessel is eventually reduced to a fibrous strand, the *ligamentum arteriosum*. While histologic obliteration may be in part dependent upon initial closure of the vessel by active constriction, in theory PDA could result from a disturbance in the mechanisms responsible for either physiologic or anatomic closure.

Knight et al. have recently studied the physiologic closure mechanism in isolated perfused ductuses from near term fetal pups genetically predisposed to PDA.[22] The ductal lumen size under conditions of hypoxia was greater than in ductuses from normal controls, and there was an absent or diminished constrictor response to oxygen, norepinephrine, and acetylcholine. In studies of the histologic architecture of the ductus arteriosus, Buchanan et al. have noted that pups from matings of dogs with hereditary PDA have a ductal wall structure which differs from that of controls of similar age.[6,23] There is a deficiency and abnormal distribution of smooth muscle cells, particularly evident in the segment of the ductus arteriosus which adjoins the aorta, and extending a variable distance toward the pulmonary artery. Elastic fibers are increased in size and number. The affected segment resembles the wall of the adjacent aorta.

Based on this evidence, it has been hypothesized that the multiple genes responsible for hereditary PDA in the dog act in some way to extend aorta-like differentiation to the ductus arteriosus.[6] According to this concept, increasing liability to PDA represents extension of the

non contractile wall structure of the aorta to an increasing segment of the ductus arteriosus, progressively impairing its capacity to undergo physiologic closure. The developmental mechanisms involved are as yet unexplored. Abnormal partitioning or differential growth of the aortic arch system may be involved.

CONGENITAL DEFECTS IN CARDIAC ACTIVATION

In man, a number of abnormalities in cardiac activation may occur in the absence of any obvious gross anatomic abnormalities of the heart. They include incomplete and complete right bundle branch block, various degrees of atrioventricular block, and the Wolff-Parkinson-White syndrome. These abnormalities have also been observed in dogs, providing an opportunity to investigate their etiology, pathogenesis, and the relationship between the alteration in cardiac activation and the body surface electrocardiogram.

Incomplete Right Bundle Branch Block (IRBBB)

This electrocardiographic pattern occurs in association with a variety of congenital and acquired cardiac lesions and occasionally is observed in patients who have no other evidence of cardiac abnormality. As the name IRBBB implies, the underlying disturbance has generally been assumed to be delayed activation of the right ventricle owing to slowed conduction along the right bundle branch or peripheral Purkinje conduction system. Recently, IRBBB (Figure 4) was discovered in a family of dogs resulting from an experimental mating between a Beagle with valvular pulmonic stenosis and a Beagle with a small subaortic interventricular septal defect (Figure 5). All seven members of the F_1 generation had varying degrees of the IRBBB pattern but no other clinical, hemodynamic or postmortem abnormalities. When mated *inter se*, these animals produced an F_2 generation in which ventricular septal defect and valvular pulmonic stenosis occurred separately and together, as well as dogs with IRBBB but no obvious gross anatomic lesions (Figure 5).

Electrophysiologic studies in six members of the F_1 generation as well as two unrelated dogs with the IRBBB pattern showed that the electrocardiographic abnormality is associated with delayed activation of the surface of the right ventricle.[8] Epicardial "break-through" of right ventricular activation in the outflow tract region was delayed beyond any points on the surface of the left ventricle, and occurred at the time of the abnormal rightwardly directed terminal QRS forces. Conduction velocity down the right bundle branch was normal. When intramural activation of the right ventricular wall was studied using plunge electrodes, it was found that the wave of activation was not delayed in reaching the endocardium of the right ventricle, but that spread through the right ventricular free wall in the region of the outflow tract was prolonged due to a localized increase in wall thickness.

The IRBBB pattern in this case was shown to result not from any abnormality in conduction through the right bundle branch, its subdivisions, or the peripheral Purkinje network, but from what appears to be a genetically determined focal variation in right ventricular wall thickness. It is of interest that no accompanying hemodynamic or structural abnormalities could be detected which would account for the hypertrophy. The localized nature of the thickened area is significant, for it points out that prolongation of the intramural spread of activation in only a small region of the right ventricle can result in changes in phase relationships of a sufficient degree to markedly alter the body surface electrocardiogram.

The Wolff-Parkinson-White Syndrome

The Wolff-Parkinson-White syndrome is an electrocardiographic abnormality characterized by an abnormally short P-R interval and a widened QRS complex having a slurred initial portion (delta wave). Affected individuals are prone to attacks of supraventricular tachycardia. It has long been known that the anomalous electrocardiographic pattern in this syndrome is the result of an accessory atrioventricular (AV) conduction pathway which allows the wave of atrial depolarization to enter the ventricle at an abnormal site, prior to arrival of the

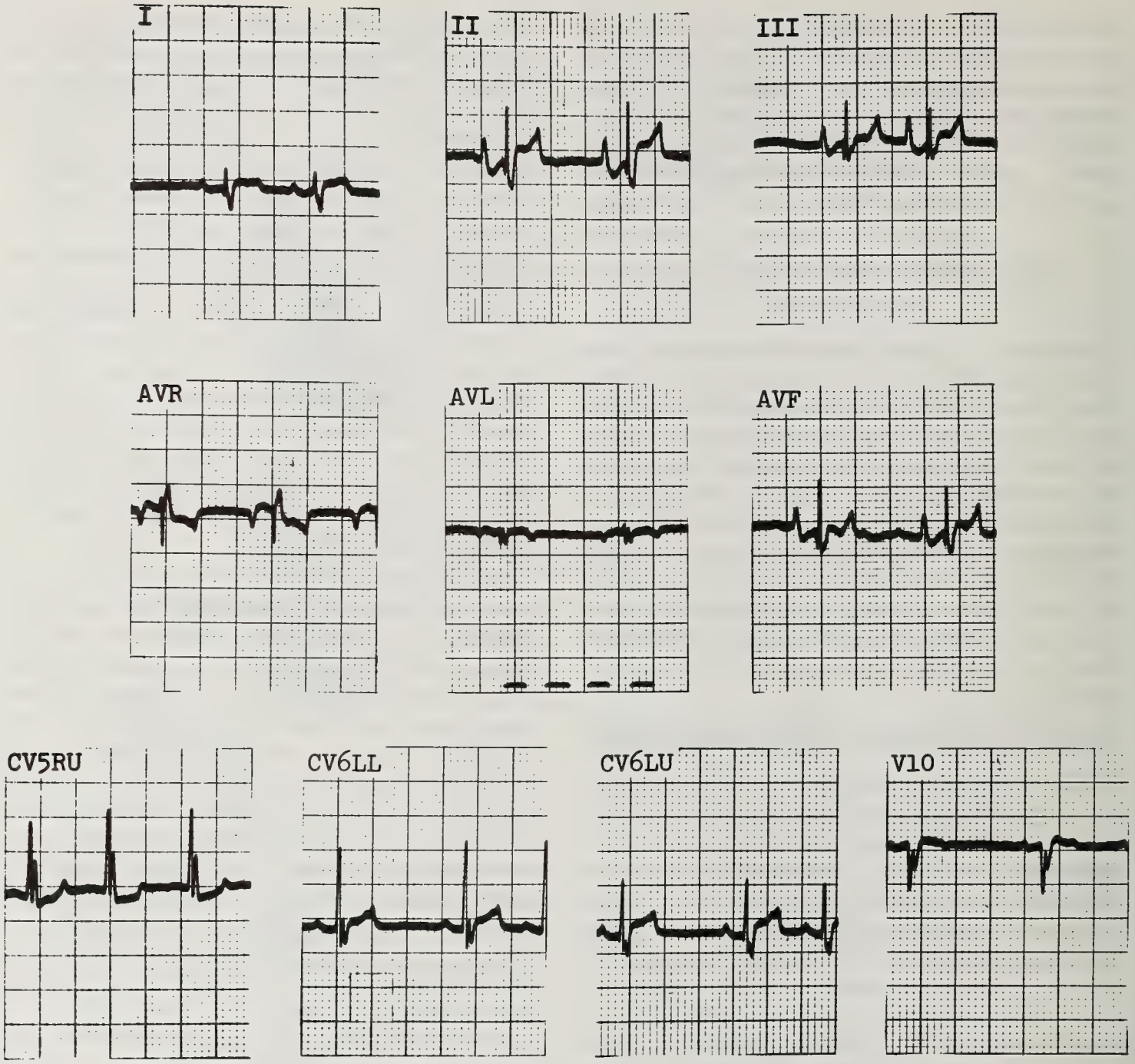


FIGURE 4.—Incomplete Right Bundle Branch Block

Ten lead electrocardiogram of a 1-year old male Beagle with incomplete right bundle branch block (No. 37 in Figure 5). The QRS interval is on the upper limit of normal for the dog (0.07 second). Terminal, delayed QRS forces are directed to the right, cranially, and sternally, producing wide, notched S waves in leads I, II, III, AVF, and the left precordial leads CV6LL (V_3) and CV6LL (V_6). An R′ wave is present in leads AVR and CV5RL (V_1).

normally conducted impulse which travels through the AV node and bundle of His.

Anomalous bridges between the atrium and ventricle have been found in patients with WPW.[24,25] Epicardial activation studies have shown an area of preexcitation of the right atrioventricular margin,[26,27] and in some cases the syndrome has been abolished by cutting the right atrioventricular margin at that site.[9,28] These findings suggest that preexcitation in WPW occurs over discrete accessory AV conduction pathways similar to those described by Kent.[29,30] However, direct electrophysiologic and anatomic evidence of preexcitation over a

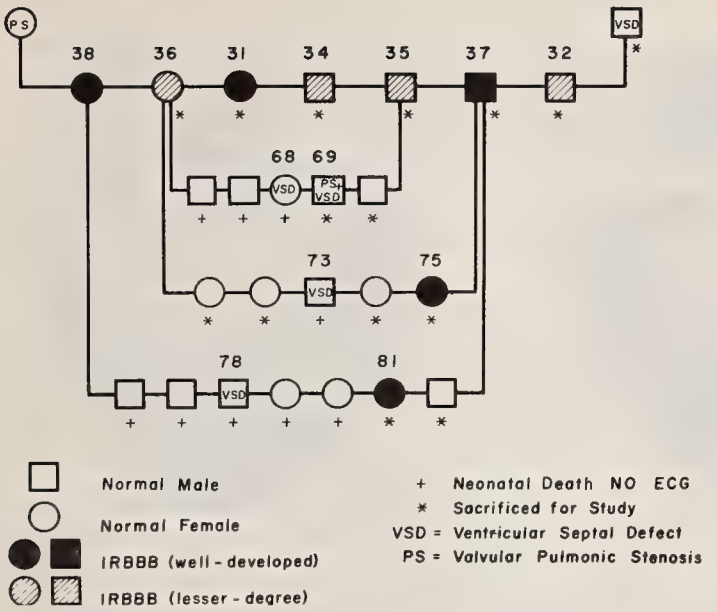


FIGURE 5.—Incomplete Right Bundle Branch Block in a Family of Beagles

A female Beagle with pulmonic stenosis and a male Beagle with a small ventricular septal defect produced 7 pups with varying degrees of incomplete right bundle branch block (IRBBB) but no gross cardiovascular malformations. Both pulmonic stenosis and ventricular septal defects appeared in the F_2 generation.

Reprinted from reference #8 with permission.

discrete atrioventricular bridge have not been reported in the same individual owing to difficulties in obtaining both electrophysiologic and anatomic data.

The WPW syndrome has also been observed in dogs. In a screening survey of cardiovascular disease in dogs presented to a veterinary clinic, it was found once among 3,000 dogs examined electrocardiographically.[31] Recently the study of an example of type A WPW syndrome in an English Cocker Spaniel dog* (Figure 6) has provided the long sought direct evidence of preexcitation of the right ventricle over a discrete AV bridge.[9] In this dog, preexcitation of the posterior (caudal) wall of the right ventricle occurred over an anomalous atrioventricular bundle having a conduction velocity and histologic structure characteristic of ventricular myocardium. Figure 7 shows sequence of epicardial activation in a dog with type A WPW syndrome.

* By remarkable coincidence, this dog belonged to Dr. Mary Livezey, cardiologist and wife of the late Dr. Charles C. Wolferth, a pioneer in electrocardiography who with Dr. Frances Wood first demonstrated the existence of an anatomic bridge between the right atrium and ventricle in a human patient with WPW.[24] Dr. Livezey's cooperation in making this dog available for study is gratefully acknowledged.

Disturbances of Atrioventricular Conduction

Relatively little is known about the etiology or pathogenesis of congenital atrioventricular blocks in the dog or man, but some interesting observations in the dog suggest that this may be a fruitful subject for further study.

James and Konde have reported extensive histologic studies of the sinus node and atrioventricular conduction system in a young dog with complete heart block.[10] The location and size of the sinus node, atrioventricular node and His bundle were normal, but the cellular architecture of these structures was distinctly abnormal. The authors hypothesize a normal embryonic origin of this specialized conduction tissue, leading to a normal spatial arrangement,

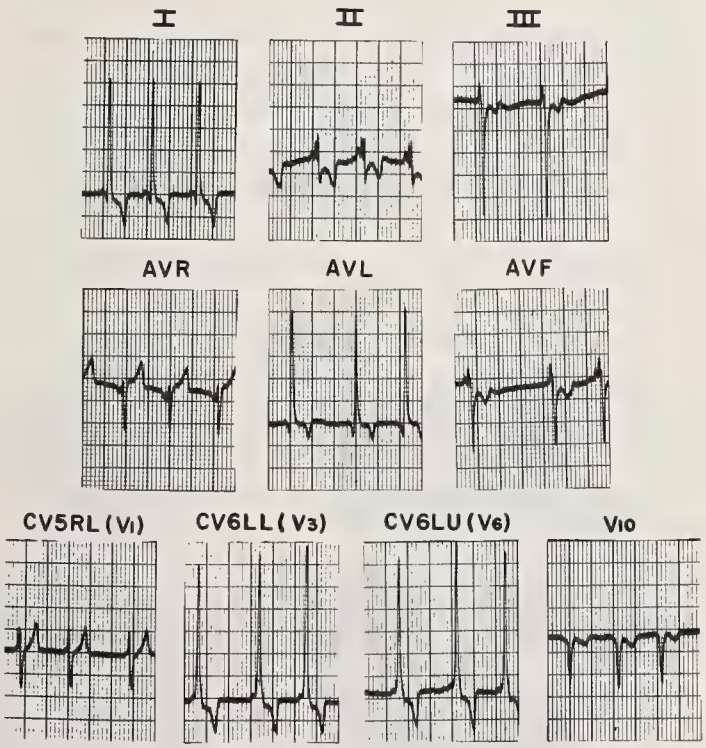
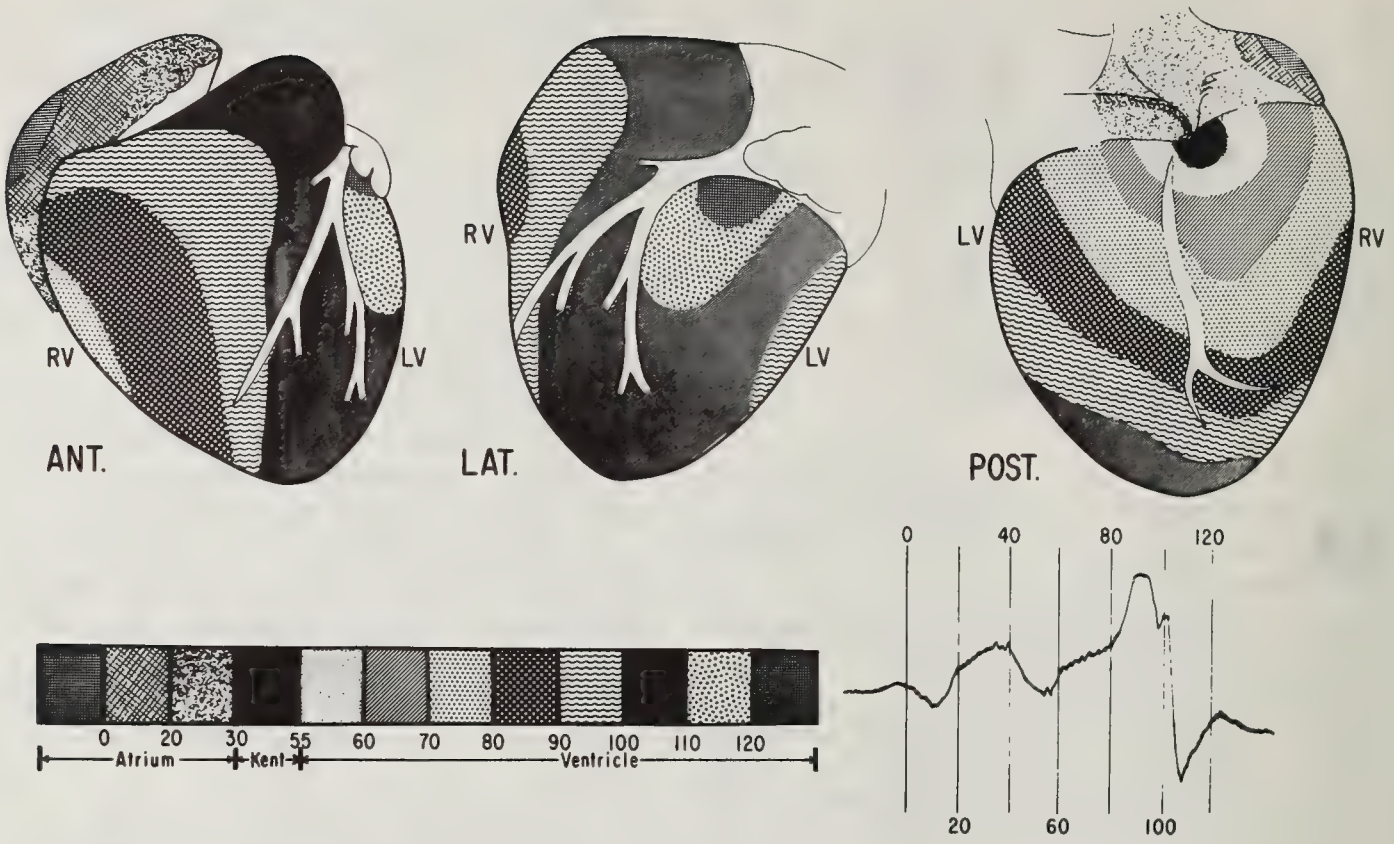
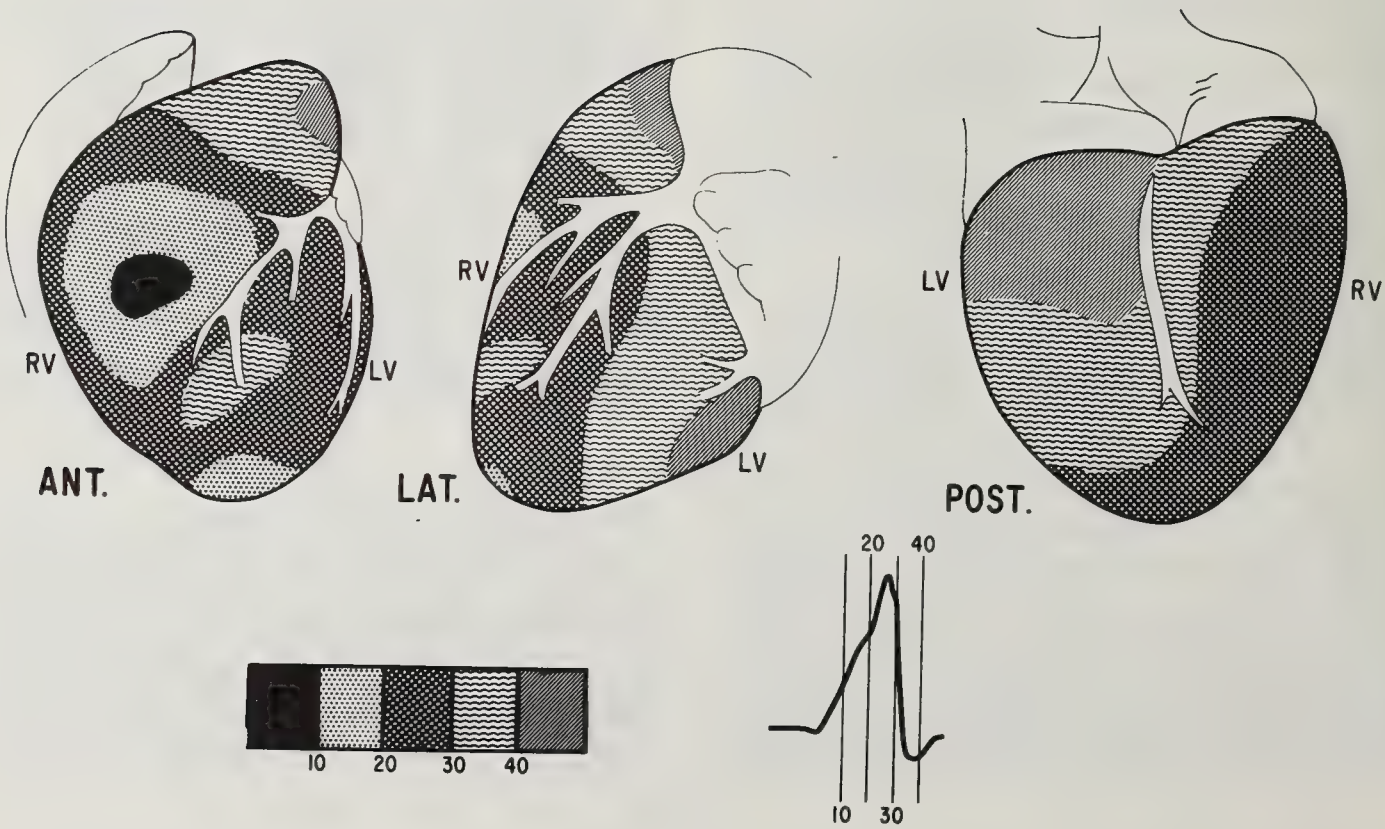


FIGURE 6.—Type A Wolff-Parkinson-White Syndrome in an English Cocker Spaniel

The P-R segment is absent in all leads and the QRS complex is prolonged. A delta wave can be seen best in leads CV6LL (comparable to V3 in man) and CV6LU (comparable to V6). Principal QRS forces are oriented to the left, toward the sternum, and cranially. Preexcitation of the right ventricle in this dog was shown to occur at the AV margin near the posterior (caudal) border of the right ventricle (9).


RV
LV
ANT.
RV
LV
LAT.
LV
RV
POST.
0 20 30 55 60 70 80 90 100 110 120
Atrium
Kent
Ventricle
0 40 80 120
20 60 100


RV
LV
ANT.
RV
LV
LAT.
LV
RV
POST.
10 20 30 40
20 40
10 30

but abnormal differentiation of the cellular elements leading to disturbance of conduction. Regretably, this dog died before electrophysiologic studies could be made. Future investigation into the etiology, pathogenesis, and electrophysiologic properties of the specialized conduction tissue in animals of this type are of great interest.

James and Drake have studied the hearts of a group of eleven Doberman Pinschers which died suddenly.[11] They found focal degeneration of the His bundle, associated with cartilage and bone formation in the adjacent central fibrous body. Small coronary arteries in the area were narrowed. Six of the eleven dogs had a common ancestor and all were purebred Doberman Pinschers. The authors have hypothesized that sudden unexpected death in these dogs may be the result of a form of Adams-Stokes attack brought about by interruption of AV conduction. If this hypothesis is correct, varying degrees of atrioventricular block might be demonstrable in early stages of the disease. Unfortunately, no electrocardiographic observations have yet been reported.

DISCUSSION AND CONCLUSIONS

Epidemiologic and genetic studies have shown that the common anatomic forms of congenital heart defects in the dog are caused by lesion-specific genetic factors which are concentrated in certain breeds. The defects so far analyzed are genetically complex. Like hereditary PDA in Poodles, most are probably polygenic "threshold" traits, having a discrete (discontinuous) phenotype, determined by a more or less continuous underlying distribution of genetic factors. Although multiple in number, the genes involved appear to affect the growth and differentiation of the developing cardiovascular system in very specific ways. The genes for a given defect may be looked upon as polygenic sets, the individual members of which have additive effects upon some underlying variable such as the concentration of some substance, or the rate of growth of some tissue element important in cardiac development. At some critical threshold value of the variable, the gross form of the developing organ becomes abnormal. In the case of hereditary PDA, available evidence suggests that the underlying genetic determinants in some way cause an extension of aorta-like differentiation to the ductus arteriosus. The threshold for PDA is reached when this quantitatively inherited defect in histodifferentiation reduces the capacity of the ductus to constrict to a level at which physiologic closure can no longer occur. As might be expected, on careful examination the abnormal phenotype is seen to exhibit a continuous gradation of severity extending below and above the threshold for PDA. The degree of severity is determined by the "dose" of genes predisposing to PDA.

The demonstration that many of the common forms of congenital heart defects in the dog are hereditary and lesion specific has a number of important implications. First, it provides the most compelling evidence yet available in an animal species that cardiovascular malformations have a primary genetic cause. Parallel evidence in man has suggested this for some time[32] but

FIGURE 7.—Sequence of Epicardial Activation in a Dog with Type A Wolff-Parkinson-White Syndrome

TOP: Epicardial surface maps, showing the sequence of activation of the anterior, left lateral, and posterior surfaces of the heart of a five year old English Cocker Spaniel bitch with Type A WPW syndrome (Figure 6). Compare with maps of a normal dog below. The times of activation are indicated in the sequence key and lead II electrocardiogram. The earliest region of epicardial activation occurred posteriorly in the right ventricle, near the septum. There was no evidence of collision (fusion) of normal and anomalously conducted wave fronts at the ventricular epicardium. Histologic sections of the region of earliest activation confirmed the existence of a muscular atrioventricular bridge.

Reprinted from reference #9, with permission of the American Heart Association, Inc.

BOTTOM: Sequence of epicardial activation in a normal dog. The format is the same as the illustration above. Note that normal excitation begins in the middle of the anterior right ventricle near the interventricular septum and spreads centrifugally and from apex to base. The posterior right ventricle, which was activated prematurely in the dog with WPW above, is activated relatively late in QRS in the normal dog.

Reprinted from reference #9, with permission of the American Heart Association, Inc.

the nature of the genetic influence is ill-defined. Although not ideal, the dog is eminently better suited for genetic investigation than man and it can be anticipated that the further study of strains of dogs having a high incidence of specific cardiovascular defects will contribute substantially to our understanding of the nature and mode of action of the genetic factors underlying congenital heart disease.

The second important implication is that dogs with predictable cardiovascular defects can now be produced for a variety of research purposes by selective breeding. They will be particularly useful in studies of the pathogenesis of cardiovascular malformations, since embryos with known defects can be obtained at any desired stage of development and used to investigate the tissue, cellular, and biochemical disturbances which lead to abnormalities of form. Previously, embryologic studies have been largely confined to normal embryos, or embryos in which defects were induced by exposure to various environmental insults. Procedures of this type usually lack the specificity necessary for the study of a particular type of developmental defect.

As was shown in congenital incomplete right bundle branch block and the Wolff-Parkinson-White syndrome, canine models may also be helpful in investigating the physiologic derangements which accompany congenital cardiovascular defects, particularly where they cannot be studied in human patients. Further examples include the study of cardiac activation in dogs with right ventricular hypertrophy accompanying congenital heart disease,[33,34] and the investigation of coronary blood flow alterations in dogs with naturally occurring subaortic stenosis.[35] In the latter case, dogs with hereditary subaortic stenosis were studied over a period of weeks after implantation of blood flow transducers, providing the only evidence yet available regarding the chronic effects of aortic stenosis on phasic coronary blood flow patterns. Although they have not yet been utilized for this purpose, dogs with congenital cardiovascular defects could be valuable in the development and testing of surgical procedures aimed at the treatment of cardiovascular malformations. They would provide a far more realistic medium than normal animals or animals in which anatomic defects have been surgically created.

REFERENCES

1. Detweiler, D. K., and Patterson, D. F. The prevalence and types of cardiovascular disease in dogs. Ann. N. Y. Acad. Sci. 127:481–516, 1965.
2. Patterson, D. F. Congenital heart disease in the dog. Ann. N. Y. Acad. Sci. 127:541–569, 1965.
3. Patterson, D. F. Epidemiologic and genetic studies of congenital heart disease in the dog. Circ. Res. 23:171–202, 1968.
4. Patterson, D. F. Canine congenital heart disease: epidemiology and etiological hypotheses. J. Small Animal Pract. 12:263–287, 1971.
5. Patterson, D. F., Pyle, R. L., Buchanan, J. W., Trautvetter, E., and Abt, D. A. Hereditary patent ductus arteriosus and its sequelae in the dog. Circ. Res. 29:1–13, 1971.
6. Patterson, D. F., Pyle, R. L., and Buchanan, J. W. Hereditary cardiovascular malformations of the dog. Birth Defects: Original Article Series, Vol. VIII, Cardiovascular System, National Foundation, in press, 1971.
7. Patterson, D. F., and Pyle, R. L. Genetic aspects of congenital heart disease in the dog. Proceedings Gaines Symposium on Newer Knowledge About Dogs. In press, 1971.
8. Moore, E. N., Boineau, J. P., and Patterson, D. F. Incomplete right bundle branch block: an electrocardiographic enigma and possible misnomer. Circulation 44:678–687, 1971.
9. Boineau, J. P., and Moore, E. N. Evidence for propagation of activation across an accessory atrioventricular connection in types A and B preexcitation. Circulation 41:375–397, 1970.
10. James, T. N., and Konde, W. N. A clinicopathologic study of heart block in a dog, with remarks pertinent to the embryology of the cardiac conduction system. Am. J. Cardiol. 24:59–71, 1969.
11. James, T. N., and Drake, E. H. Sudden death in Doberman Pinchers. Ann. Int. Med. 68:821–829, 1968.
12. Parker, G. W., Jackson, W. F., and Patterson, D. F. Coarctation of the aorta in a canine. J.A.A.H.A. 7:353–355, 1971.
13. Hay, J. D. Population and clinic studies of congenital heart disease in Liverpool. Brit. Med. J. 2:661–667, 1966.
14. Mulvihill, J. J. Comments on the epidemiology of congenital heart disease in dogs. Birth Defects: Original Article Series, Vol. VIII. Cardiovascular System. National Foundation, 1971. (Paper presented at the 4th Conference on the Clinical Delineation of Birth Defects, Baltimore, Md., June, 1971).
15. Rudolph, A. M., Auld, P. A. M., Golinko, R. J., and Paul, M. H. Pulmonary vascular adjustments in the neonatal period. Pediatrics 28:28–34, 1961.

16. Phillips, C. E., DeWeese, J. A., Manning, J., and Mahoney, E. B. Maturation of small pulmonary arteries in puppies. Circ. Res. 8:1268–1273, 1960.
17. Rudolph, A. M. The changes in the circulation after birth: Their importance in congenital heart disease. Circulation 41:343–359, 1970.
18. Born, G. V. R., Dawes, G. S., Mott, J. C., and Rennick, B. R. The constriction of the ductus arteriosus caused by oxygen and asphyxia in newborn lambs. J. Physiol. (London) 132:304–342, 1956.
19. Kovalcik, V. The response of the isolated ductus arteriosus to oxygen and anoxia. J. Physiol. (London) 169:185–197, 1963.
20. Assali, N. S., Morris, J. A., Smith, R. W., and Manson, W. A. Studies on ductus arteriosus circulation. Circ. Res. 13:428–489, 1963.
21. Melmon, K. L., Cline, M. J., Hughs, T., and Nies, A. S. Kinins: Possible mediators of neonatal circulatory changes in man. J. Clin. Invest. 47:1295–1302, 1968.
22. Knight, D. H., Patterson, D. F., and Melbin, J. Constriction of the fetal ductus arteriosus in normal dogs and those genetically predisposed to persistent patency. Circulation 64:114. (Supplement II, Abstracts 44th Scientific Sessions, American Heart Association, 1971).
23. Buchanan, J. W., Patterson, D. F., and Pyle, R. L. Morphologic studies in dogs with hereditary patent ductus arteriosus. Circulation 64:147 (Supplement II, Abstracts 44th Scientific Sessions, American Heart Association, 1971).
24. Wood, F. C., Wolferth, C. C., and Geckeler, G. D. Histologic demonstration of accessory muscular connections between auricle and ventricle in a case of short P-R interval and prolonged QRS complex. Am. Heart J. 25:454–462, 1943.
25. Lev, M., Gibson, S., and Miller, R. A. Ebstein's disease with Wolff-Parkinson-White syndrome. Am. Heart J. 49:724–741, 1955.
26. Durrer, D., and Roos, J. P. Epicardial excitation of ventricles in a patient with Wolff-Parkinson-White syndrome (Type B). Circulation 35:15–21, 1967.
27. Burchell, H. B., Frye, R. L., Anderson, M. W., and McGoon, D. C. Atrioventricular and ventriculoatrial excitation in Wolf-Parkinson-White syndrome (Type B). Circulation 36:663–672, 1967.
28. Cobb, F. R., Blumenschein, S. D., Sealy, W. C., Boineau, J. P., Wagner, G. S., and Wallace, A. G. Successful surgical interruption of the bundle of Kent in a patient with Wolff-Parkinson-White syndrome. Circulation 38:1018–1029, 1968.
29. Kent, A. F. S. Observations on auriculo-ventricular junction of mammalian heart. Quart. J. Exp. Physiol. 7:193–195, 1913–1914.
30. Kent, A. F. S. The structure of the cardiac tissues at the auriculoventricular junction. J. Physiol. 47:17–28, 1913–1914.
31. Patterson, D. F., Detweiler, D. K., Hubben, K., and Botts, R. P. Spontaneous abnormal cardiac arrhythmias and conduction disturbances in the dog: A clinical and pathological study of 3,000 dogs. Am. J. Vet. Res. 22:355–369, 1961.
32. Nora, J. J. Multifactorial inheritance hypothesis for the etiology of congenital heart disease. Circulation 38:604–617, 1968.
33. Hill, J. D., and Moore, E. N. Epicardial excitation studies in dogs with congenital right ventricular hypertrophy. Circulation Res. 20:649–657, 1967.
34. Boineau, J. P., Hill, J. D., Spach, M. S., and Moore, E. N. Basis of the electrocardiogram in right ventricular hypertrophy: Relationships between ventricular depolarization and body surface potentials in dogs with spontaneous RVH contrasted with normal dogs. Am. Heart J. 26:605–627, 1968.
35. Pyle, R. L., Lowensohn, H. S., Khouri, E. M., Gregg, D. E., and Patterson, D. F. Coronary artery hemodynamics in dogs with congenital subaortic stenosis. Circulation 64:115. (Supplement II, Abstracts 44th Scientific Sessions American Heart Association, 1971).

ACKNOWLEDGMENT

The author is grateful to his colleagues of the Comparative Cardiovascular Studies Unit, University of Pennsylvania, for their past and continuing contributions to the studies described in this paper. He is particularly indebted to Dr. D. K. Detweiler, Dr. R. L. Pyle, Dr. J. W. Buchanan, Dr. D. H. Knight, and Dr. E. N. Moore.

DISCUSSION

Chairman Pierce: Are there any questions for Dr. Patterson?

Dr. Patterson, we are all aware of your valuable resource, of not only congenital patent ductus, but other lesions as well. What is the practicality of other laboratories developing colonies of such animals?

Dr. Patterson: Well, certainly, our experiments which yielded from 10 to 90% affected offspring, show that it is quite feasible to produce animals with cardiovascular defects for a variety of different purposes. One intriguing

thought, which has occurred to me, is that many surgical procedures developed for the treatment of congenital heart disease in man are done in normal animals. It would be very nice indeed for cardiac surgeons and others involved in this area to have truly anatomical replicas of what it is they're trying to correct, not only to develop the operations, but to test their efficacy.

SPONTANEOUS DISEASES IN GERMFREE ANIMALS*

Morris Pollard*

Germfree rats and mice have been propagated and maintained in colony form during the past 12 years. The methodology, as evolved during the past 35 years, has been so standardized and simplified that research programs can now be implemented with them in almost any biological laboratory. Germfree animals are comparable to the conventional counterpart animals from which they had been derived, as regards reproduction patterns, litter sizes, growth rates, and appearances. The bacteria-free status of germfree animals is associated with a dilated, thin-walled, intestinal tract, and relatively inactive immunogenic mechanisms. Except for mice with congenital diseases (viral and non-viral), the spontaneous diseases encountered in germfree mice and rats have been of relatively low incidence, and their longevity has been extended. Germfree animals are free of "horizontally"-transmitted infectious agents. Three "vertically"-transmitted viruses, and associated diseases, have been observed among germfree mice: leukemia, mammary tumor, and lymphocytic choriomeningitis viruses. A unique spontaneous "wasting" disease has been observed in germfree AKR mice. Thus far, no viral agent has been detected among germfree rats; however, as they aged they manifested, predominantly, increased numbers of adenomas in endocrine organs. Malignant tumors among them have been very rare. None of the classical tissue lesions, described in aged conventional rats, has been observed in the germfree rats. Germfree rats and mice are susceptible to experimental diseases resulting from exposures to chemical, physical, and biological agents. The syndromes thus induced in germfree animals have been more clearly defined because of their isolation from the complicating effects of irrelevant agents from the environment. They are excellent subjects for biomedical research.

INTRODUCTION**

The evolution of germfree technology during the past 70 years has resulted in the development of unique and defined experimental animals and associated instruments for clinical applications.[1-7] Germfree rats and mice have been subjected to extensive examinations during the past ten years, as regards susceptibility to, and manifestations of, spontaneous and induced diseases. Viral agents have been found in germfree mice which necessitated coinage of the designation "gnotobiotic" (of known content) to identify them. At the present time, there is no decisive evidence of a microbial flora in germfree rats; so that they are considered technically as germfree or axenic. For convenience they are referred to here as "germfree." This report concerns spontaneous diseases in germfree mice of seven strains and in germfree rats of three strains that have been propagated and maintained in this laboratory during the past 10 years (Table I).

* Lobund Institute, University of Notre Dame, Notre Dame, Indiana.

** Supported by funds from U.S. Public Health Service Grant RR00294 and the John A. Hartford Foundation.

CHARACTERISTICS OF GERMFREE MICE AND RATS

Germfree rats and mice were derived from conventional mothers by caesarian delivery of fetuses from the uterus directly into a sterile plastic or steel container which is referred to as an isolater.[4,6] The original babies were fed a sterile liquid diet by stomach tube up to weaning age; and thereafter, they sustained themselves on a sterile solid diet and water. They have been maintained and propagated continu-

TABLE I.— *Strains of Germfree Mice and Rats at Lobund Institute*

	Strain	Source	Generation in germfree status
Mice:			
	Swiss-Webster	Harlan Farms	45
	CFW	A. Werder	36
	C3h/f	H. B. Andervont	16
	Balb/c	H. B. Andervont	27
	AKR	John Trentin	28
	Haas	Wallace P. Rowe	22
	SJL/J	Sam Poiley	8
Rats:			
	Wistar	Wistar Institute	30
	Sprague-Dawley	Charles River Laboratories	21
	Fischer (inbred)	Andrew Schmidt	7

ously in the sterile isolator system with sterile water, food, bedding, and air. The diet now recommended for germfree rats and mice is L485,[8] which has been tested and monitored with satisfactory results. The rats and mice are free of demonstrable parasites, protozoa, fungi, and bacteria. A standard bacteriological monitoring system has been developed which combines accuracy with simplicity.[9]

"Vertically-transmitted" viruses (as described later) have been demonstrated in the mice, but not yet in the rats. Tests for viral flora have involved examinations of target organs (thymus) for virus-like particles by electron microscopy, serological surveys, examinations of tissue cultures derived from fetuses and of tumors for cytopathogenic effects, viral inclusions, and susceptibilities to known viral agents.[10-12] It is significant that so-called "horizontally"-transmitted viruses have not been detected in germfree mice,[12] nor have they been detected in germfree rats. Under such circumstances, the rats and mice reach maturity, reproduce, and grow old. New strains of germfree mice and rats are usually foster-nursed on germfree mothers up to weaning age, and thereafter they are self-sustaining. The germfree technology has been so simplified that short-term experiments can be implemented in most laboratories with mice and rats in equipment acquired from commercial sources.[14]

Germfree rats and mice have some unique anatomical and physiological characteristics which distinguish them from conventional counterpart animals. The intestines and cecums are thin-walled and dilated, and the lamina propriae are small, thin, and contain relatively few cells. The turnover rate of mucosal cells in the intestine is lower than that in conventional animals.[15] The Peyer's patches are usually small aggregations of lymphocytes, and free of germinal zones. The lymph nodes and spleens are small, contain rare germinal zones, and few plasma cells. The immunological mechanisms in germfree animals are dormant, but functionally intact; and this is reflected in the low levels of serum globulins. Following exposures to antigens, they enlarge, and germinal zones and plasma cells appear in them. White blood cell counts and blood hematocrit levels are lower in germfree rats and mice than in conventional counterpart animals.[13] The characteristic pattern of enlargement and involution of the thymus glands in germfree and in conventional rats and mice are undistinguishable. In general, germfree animals are comparable to conventional animals in reproduction patterns, growth rates, and appearance; and whatever differences they manifest are related to the effects of variable microbial flora and other non-viable environmental factors to which the conventional animals had been exposed.

DISEASE IN GERMFREE MICE AND RATS

Most germfree mice and rats are free of spontaneous diseases up to ages 6 and 12 months, respectively. Except for mouse strains SJL/J, NZB, Haas, and AKR, the longevities of germfree mice and rats have been extended significantly through their unique insulated residence. The patterns of spontaneous diseases in germfree mice differ from those in germfree rats. Germfree mice carry congenitally-transmitted viruses which are life-limiting in some strains (AKR and Haas); and in other strains (NZB and SJL/J) fatal diseases develop spontaneously for which etiological agents have not been identified.[16-19] In many cases, the differences between the diseases in conventional and in germfree rodents are attributed to environmental factors which the germfree system has been designed to exclude, but this cannot be applied to mice with congenitally-acquired diseases (viral or genetic).

SPONTANEOUS DISEASES IN GERMFREE MICE

Thus far, three virus-related and two genetic-related diseases have been observed in germfree mice:

Viral Diseases

Leukemogenic viruses have been observed in all strains of mice (conventional and germfree);[20] however, among the seven strains of germfree mice at Lobund Laboratory, only the AKR strain develops lymphatic leukemia

TABLE II.—*Lesions observed in Female Germfree Mouse Strains*

	CFW	C3H	Balb/c	Swiss-Webster
Number examined	32	55	19	39
Lymphatic leukemia	1 (4)*			1 (3)
Breast carcinoma	2 (X, 23)	5 (4, 10, 10, 16, 17)	1 (13)	4 (5, 5, 11, 11)
Fibrosarcoma	2 (7, 18)			1 (5)
Lymphosarcoma	1 (18)			
Osteosarcoma				1 (6)
Fibroma	1 (9)			
Amyloidosis	1 (23)			
Negative	24**	50 (6)	18 (14)	32 (10)

* Number of mice with lesions (age in months).

** Negative: 17 females (11, 11, 11, 11, 11, 12, 12, 12, 13, 14, 14, 18, 18, 25, 25, 25, 26); 7 males (12, 12, 13, 13, 14, 14, 23).

X—not recorded.

spontaneously and in significant numbers. In the course of ten years, only 2 mice, from among the remaining strains, have developed lymphatic leukemia spontaneously (Table II); but leukemia can be induced in all strains by administrations of whole-body x-irradiation.[21] Germfree and conventional AKR mice manifest the same incidence (over 95%) and type of disease, at average age of 8 months; and all of them die within 7 days after onset of symptoms. Occasionally, younger germfree AKR mice have developed spontaneously what appears to be a "wasting" syndrome which has been interpreted possibly as an early manifestation of leukemia.[22] They appear kyphotic and rough and are disinclined to move. In such mice, the thymus glands are involuted; but the spleens and lymph nodes are swollen with cords of plasma-type cells. They usually manifest perianal dermatitis and diarrhea, and they usually die within 7 days after onset of symptoms. This "wasting" syndrome has not been observed in conventional AKR mice nor in germfree mice of other strains.

Spontaneous breast carcinomas have been observed in four strains of germfree mice (Table II), and "B"-type virus particles (as well as "C"-type particles) have been noted in the tumors of three strains by electron microscopy.[23,24] In spite of this obvious virus contamination, the incidence of spontaneous breast carcinomas among them has been relatively low. This may be related to standards of husbandry. The data recorded in Table II fails to set an accurate incidence level of breast tumors because candidate mice had been withdrawn continuously from the breeding colonies for other studies, which were often terminated before the mice had reached 6 months of age. It is significant to note here, and as recorded earlier,[23,24] that germfree mice do carry "B"-type virus particles. A confirmatory report on germfree Balb/c mice indicates that their tumors are similar to those observed in conventional mice.[25]

A mouse strain (designated "Haas") with congenitally-transmitted lymphocytic choriomeningitis virus has now been propagated in germfree status through 22 generations.[17] All of them have shown a life-long viremia (10^5 LD_{50}/ml blood). They remain disease-free until age 5 months, and then small microscopic accumulations of lymphoid cells appear in the visceral organs, which expand with age. The swollen lymph nodes and spleens contain large germinal zones and masses of lymphoid cells, many of them plasma cells. The serum globulin levels become elevated; and degenerative lesions appear in the glomeruli.[26] The glomerular vessels become occluded by accumulations of fibrinoid material;[27] and eventually the glomeruli become hyalinized, sclerosed and obliterated. Glomerulonephritis is a classical lesion in this mouse strain. Arteritis has been described in them.[28] The Haas mice usually show clinical evidence of disease at 9 months, and they die about 12 months of age. Their visceral organs are distorted and infiltrated extensively with lymphoid cells which are assembled to form plasmacytomas and reticulum cell sarcomas. Immunoproliferative lesions develop also in conventional Haas strain mice, but their lesions are not so precisely defined because of complicating bacterial infections. The passage of LCM virus in Haas strain mice via transovarial and/or transplacental routes is now being determined.[29]

Genetic Diseases

Two strains of germfree mice (NZB and SJL/J) have manifested the same disease syndromes as the conventional stock from which they had been derived.[18,19] While leukemia-related "C"-type virus particles have been observed in them, their etiological relationships to

the unique lesions of hemolytic anemia and reticulum cell sarcoma, respectively, have not been clarified, and the pathogenesis of both syndromes appear to be regulated by genetic mechanisms.

Aside from the diseases noted above, sebaceous cysts, hernias, and neoplasms have been observed occasionally in other strains of germfree mice (Table II); however, the great majority of them live without apparent disease. Amyloidosis has been observed rarely in Lobund strains of germfree mice. While most strains of germfree mice are rarely handicapped by the enlarged cecum, this laboratory has the impression that this enlarged organ may be responsible for the poor reproduction records of C57 BL and SJL/J mice that exist in this Laboratory. *Eperythrozoon coccoides* has been detected in the blood cells of commercially-available strains of germfree mice;[30] but an extensive survey has failed to reveal this organism in Lobund germfree mice.[31]

SPONTANEOUS DISEASES IN GERMFREE RATS

A survey of aged germfree Lobund Wistar rats (>24 months) in 1962 revealed that many of them were afflicted with nephrosis, plus some benign and malignant breast tumors.[32] In a subsequent survey of aged germfree rats, neither nephrosis nor malignant neoplasms were found among them.[33,34] Their tumors were benign and localized, and involved predominantly endocrine-related organs and occasional lipomas (Table III). In order of increasing frequency, the organs involved with benign tumors have been thyroid, thymus, liver, pituitary, breast, and adrenal glands. The percentage of rats with detectable tumors increased from 0 at age 12 months, 16 at 15 months, 30 at 20 months, 56 at 25 months to 77 at age 30 months. In one male germfree Wistar rat killed at age 31 months, benign adenomas were observed in the thymus, adrenal, pituitary, liver, and thyroid glands. In a second male germfree Wistar rat, killed at the same age, benign adenomas were observed in the pituitary and adrenal glands.

The lymph nodes of aged germfree rats contain rare germinal zones, but unusual cords of plasma cells have been observed in the medullary areas. The data recorded in Table III would suggest that tissue lesions appear in germfree rats at or after 25 months of age. This might be misleading, except for those lesions which were visible externally (i.e., tumors of the breast). Rats with large pituitary tumors have had locomotor difficulties, but the other tumors induced no significant clinical manifestations. Specified tissue lesions have been recorded by others as "hallmarks" of the aging rat: they included nephrosis, degeneration of cardiac and skeletal muscles, and polyarteritis.[35,36] In addition to the data recorded on Wistar rats in Table III, the so-called aging lesions have not yet been observed in germfree Sprague-Dawley and Fischer strain rats (Table IV).

TABLE III.—*Spontaneous lesions in Germfree Wistar rats*

	Age/months*					
	12 ± 2	15 ± 2	20 ± 2	25 ± 2	30 ± 2	Total
Number	36	12	10	60	13	131
Sex: Male-Female	25–13	2–10	10–0	37–23	10–3	82–49
No lesions: Male-Female	23–13	0–10	7–0	19–18	2–1	51–42
Monocytic leukemia					1–0	1
Adenomafibroma of breast				$7^{(5)}$–2	$2^{(1)}$–0	11
Adenoma – adrenal		$1^{(1)}$**–0	3–0	10–2	$4^{(1)}$–0	18
pituitary		1–0		2–1	4–2	10
thymus				1–3	$2^{(2)}$–0	6
liver				2–0	$4^{(1)}$–0	6
thyroid				1–1	2–0	4
lung		1–0		1–0		2
Lipoma				$3^{(1)}$–0	0–$1^{(1)}$	4
% of rats with tumors	0	16	30	56	77	62

* One rat with fibrosarcoma has been observed at age 7 months.
** (1) indicates number of rats with more than one lesion.

TABLE IV.—*Lesions in Germfree Rats*

Strain	No.	Age/ months	Sex Males	Sex Females	Lesions
Sprague-Dawley	1	8		1	Adenofibroma of breast
	1	12		1	- - -
	6	13		6	- - -
	3	14		3	- - -
	1	17	1		Fibroma
	2	18	2		- - -
	6	19	2	4	2 with pituitary tumors
	20		5	15	
Fischer	2	11		2	- - -
	18	12		18	- - -
	20			20	

Among 20 germfree Sprague-Dawley rats that were killed for examinations, benign adenomas were observed in 3 of them; but none was detected among 20 germfree Fischer rats at age 11 and 12 months. In these, as in the larger sample of germfree Wistar rats, other tissue lesions could not be detected that have been observed in aging conventional rats, such as pneumonia and nephrosis.[35,36] The absence of kidney lesions in the germfree rats has been attributed to an all-vegetable diet (L-485) on which the rats have been fed since 1964.[8] Tests on urines from individual aged germfree rats have revealed protein levels of less than 5 mg%, which reflected the healthy functional and morphological status of their kidneys, which was in sharp contrast to nephrotic tissue changes and proteinuria up to 50 mg% in conventional counterpart rats. Liver xanthine oxidase levels and bone marrow mineral components remained at high levels in aging germfree rats; whereas they drifted down in the tissues of conventional counterpart rats.[37] There is no evidence of malnourishment in the germfree rats which might explain the low incidence of diseases in them. The changes observed in the tissues of conventional rats may reflect a manifestation of precocious clinical senescence.

Chronic pneumonia of rats is considered a "horizontally-transmitted" infectious disease. It has not been observed in germfree rats. The lung tissues of germfree rats contain no focal accumulations of lymphoid cells in the peribronchial regions. The gastro-intestinal tracts of germfree rats are thin-walled and enlarged, and the Peyer's patches are small and relatively inactive. The enlarged cecum has had little pathogenic effect in Lobund germfree rats.

The spontaneous diseases of germfree rats can be characterized more by those that are absent, than present. The number of Wistar rats examined in excess of age 20 months, has now reached 83. Except for 2 of them, all were killed as a means of reducing the size of the colony; so it is not known how long 50% of them will live, nor of what they will die. The 50% survival time among the conventional Wistar rats was 18 months. Two germfree rats have died within recent months: one (age 7 months) carried a localized fibrosarcoma and the other at age 30 months had monocytic leukemia. Causes of these lesions are unknown.

DISEASES INDUCED IN GERMFREE RATS

A broad spectrum of malignant neoplastic lesions have been induced experimentally in germfree rats by chemical, physical, and biological agents (Table V).[34,38] Except from those rats which had been inoculated with Gross leukemia or Rous sarcoma viruses, no viral agent has been recovered from the rats, nor from the tumor tissues that they carried. The development of malignant breast carcinomas in the germfree rats of 1963 may be diet-related,[32] since those which were on diet L485 were free of such lesions. The data are no more than suggestive. Germfree animals have tolerated larger doses of the immunosuppressive and cytotoxic drug, cyclophosphamide, than their conventional counterparts which usually died of secondary infections.[39,40] Also, germfree rats

TABLE V.—*Experimental Induction of Lesions in Germfree Rats*

Agent	Route	Lesion induced
Methylcholanthrene	intramuscular	fibrosarcoma 52
7, 12 dimethylbenzanthracene	oral	mammary carcinoma 53
7, 12 dimethylbenzanthracene	intraperitoneal	myelogenous leukemia 54
x-irradiation	whole-body	mammary carcinoma 55
x-irradiation	whole-body	multiple degenerative lesions 55
Rous sarcoma virus	subcutaneous	fibrosarcoma 56
Gross leukemia virus	intraperitoneal	lymphatic leukemia 57
Polyoma virus	intraperitoneal	fibrosarcoma, nephroma, osteoma 58

have manifested lesions resulting from specific nutritional deficiencies,[41] except that they can tolerate acute deficiencies of vitamins A[42] and C. A small number of germfree Wistar rats, fed a standard diet supplemented with 5% sucrose in water, developed cardiovascular lesions which were absent in control rats on the same diet, but without sucrose water.[43] Germfree rats were free of dental caries, unless they had been maintained on sucrose water and contaminated with a cariogenic bacterium such as enterococci.[44] Germfree rats should provide unique advantages for determining the pathogenic potential of dietary sucrose.

DISEASE CONTROL IN GERMFREE ANIMALS

The incidences of spontaneous diseases in germfree mice have been relatively low; but it has been impossible to control the specific strain-related virus-induced diseases in AKR and Haas mice. In the latter strains, as in strains NZB and SJL/J, the disease manifestations were not modified by the germfree status of the host. The diseases recorded in the 4 mouse strains noted above constitute definite life-limiting factors. The development of spontaneous malignant tumors in mice limits their value as experimental subjects. In addition, the universal "C"-type virus infection in all mice thus far examined confuses their causal relationships to chemical-induced neoplasms by appearing in or on the tumor cells as "passengers."[45] Germfree mice have few diseases unique to their germfree status. The only strain-related condition thus far detected is the spontaneous "wasting," a disease of germfree AKR mice. All others are related to congenitally-controlled disease mechanisms which appear in both germfree and conventional stock. Germfree mice and rats live longer than their conventional counterparts[46,47] and are spared the disease complications derived from the exogenous bacterial flora. The great benefits in most strains of germfree mice relate to the low level of spontaneous diseases that they develop in a controlled gnotobiotic environment and to their survival following such procedures as neonatal thymectomy,[48] whole-body x-irradiation,[49,50] immunosuppression by drug actions[39,40] and radiation-induced bone marrow chimerism.[51] The enlarged cecum may be considered abnormal, but it is infrequently a disease factor in most strains of mice.

In contrast to mice, no infectious agent has, as yet, been detected in germfree rats. They develop, spontaneously, benign adenomas of endocrine-related organs which may eventually turn malignant, although there is no evidence to substantiate this. The lesions in the endocrine organs may conceivably produce a physiological dyshomeostasis, as a preamble to aging.[49] The absence of the "classical" lesions of aging in the germfree rats may reflect their insulation from cell-depleting toxic factors in the environment, with resultant sparing of stem cells which have repair or replacement functions. Since the strains of Lobund germfree rats do not have detectable congenital infections, the other modifying factors in their environment could come from diet, humidity, temperature, crowding, light, and noise. These have been controlled and rendered uniform by current practices of germfree technology. In view of their low profile of spontaneous diseases, their longevity, the controlled environment in which they live, and their susceptibilities to induced diseases, germfree rats are excellent, versatile, experimental subjects for bimedical research.

SUMMARY

The origins and characteristics of germfree mice and rats have been reviewed. Genetically-governed diseases in mice due to congenitally-transmitted viruses and to diseases of unknown etiology have been described. Three congenitally-acquired, life-limiting, virus infections have been described in germfree (gnotobiotic) mice, which cause specific diseases in three genetic strains of mice. Two strains of germfree mice (NZB and SJL/J) develop spontaneous diseases of unknown etiology, which usually kill them by age 12 months. Germfree rats are free of detectable microbial agents. As they grow older they spontaneously develop benign neoplasms which affect predominantly endocrine organs. Germfree rats at age 24 months do not develop the classical lesions of aging which have been described in conventional rats. While they

live longer than conventional rats, their longevity and causes of death are unknown at this time. Their susceptibilities to experimentally induced diseases make them excellent instruments for biomedical research programs.

REFERENCES

1. SCHOTTELIUS, M. Die bedeutung der darmbakterien fur die ernahrung. II. Arch. Hyg. 42:48, 1902.
2. COHENDY, M. Experiences dur la vie sans microbes. Ann. Inst. Pasteur 26:106, 1912.
3. GLIMSTEDT, G. Das leben ohne bakterien. Sterile Aufziehung von Meerschweinchen. Anat. Anz., Erganz-heft 75:79, 1932.
4. REYNIERS, J. A. Germfree research: A basic study in host-contaminant relationship. I. General theoretical aspects of the problem. Bull, N. Y. Acad. Med. 31(3):231, 1955.
5. GUSTAFSSON, B. Germ-free rearing of rats. General technique. Acta Pathologica et Microbiologica Scandinavica. Supp. LXXIII, 1948 (Doktorsav-handling-Thesis).
6. TREXLER, P. C., and REYNOLDS, L. I. Flexible film apparatus for the rearing and use of germfree animals. Appl. Microbiol. 5:406, 1957.
7. TEAH, B. A. (Ed.). *Bibliography of Germfree Research (1963–1969)*, Lobund Laboratory, Notre Dame, Indiana.
8. KELLOGG, T. F., and WOSTMANN, B. S. Stock diet for colony production of germfree rats and mice. Lab. Animal Care 19(6):812, 1969.
9. WAGNER, MORRIS. Microbiological monitoring in gnotobiotes. National Acad. of Sciences (U.S.) p. 28–39, 1970.
10. POLLARD, M. The use of germ-free animals in virus research. Progr. Med. Virol. 7:362–376, 1965.
11. POLLARD, M. Viral status of "germ-free" mice. Nat. Cancer Inst. Monograph No. 20, p. 167, 1965.
12. PARKER, J. C., TENNANT, R. W. and WARD, T. G. Virus studies with germfree mice. I. Preparation of serologic diagnostic reagents and survey of germfree and monocontaminated mice for indigenous murine viruses. J. Nat. Cancer Inst. 34:371, 1965.
13. POLLARD, M. The Germfree Rat. *Pathobiology Annual.* Ed. H. L. Ioachim, Appleton-Century-Crofts, N. Y. pp. 83–94, 1971.
14. POLLARD, M. Applications of germfree animals to problems in comparative medicine. *Adv. Veter. Sci.* Eds. C. A. Brandley & C. Cornelius, Academic Press, New York, 11:139, 1967.
15. ABRAMS, G. D., BAUER, H., and SPRINZ, H. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. Lab. Invest. 12(3):355–364, 1963.
16. POLLARD, M., KAJIMA, M., and TEAH, B. A. Spontaneous leukemia in germ-free AKR mice. Proc. Soc. Exp. Biol. Med. 120(1): 72, 1965.
17. POLLARD, M., SHARON, N., and TEAH, B. A. Congenital lymphocytic choriomeningitis virus infection in gnotobiotic mice. Proc. Soc. Exp. Biol. Med. 127:755–761, 1968.
18. EAST, J., PROSSER, P. R., and HOLBOROW, E. J. Autoimmune reactions and virus-like particles in germfree N.Z.B. mice. Lancet 1:755–757, 1967.
19. SEIBERT, K., and POLLARD, M. (Unpublished information) 1971.
20. KAJIMA, M., and POLLARD, M. Wide distribution of leukaemia virus in strains of laboratory mice. Nature 218:188, 1968.
21. POLLARD, M., and KAJIMA, M. Radiation-induced leukemia in germfree rodents. Proc. Int. Conf. Rad. Biol. and Cancer, Kyoto, Publ. Rad. Soc., Japan, p. 1, 1967.
22. POLLARD, M. Spontaneous "secondary" disease in germfree AKR mice. Nature 222(5188):92–94, 1969.
23. KAJIMA, M., and POLLARD, M. Detection of virus-like particles in germ-free mice. J. Bacteriol. 90(5):1448–1454, 1965.
24. POLLARD, M. Germ-free animals in cancer research. Gann Monograph No. 5: *Experimental Animals in Cancer Research.* Maruzen Co., Ltd., Tokyo, p. 37–43, 1968.
25. SMITH, C. S., and PILGRIM, H. I. Spontaneous neoplasms in germfree Balb/c P. mice. Proc. Soc. Exp. Biol. and Med. 138:542–544, 1971.
26. POLLARD, M., and SHARON, N. Immunoproliferative effects of lymphocytic choriomeningitis virus in germfree mice. Proc. Soc. Exp. Biol. Med. 132(1):242–246, 1969.
27. KAJIMA, M., and POLLARD, M. Ultrastructural pathology of glomerular lesions in gnotobiotic mice with congenital lymphocytic choriomeningitis (LCM) virus infection. Am. Jr. Path. 61:117–130, 1970.
28. KAJIMA, M., and POLLARD, M. Arterial lesions in gnotobiotic mice congenitally-infected with LCM virus. Nature 224 (5215):188–190, 1969.
29. SHARON, N., and POLLARD, M. (Unpublished Information) 1972.
30. TOIVANEN, A. (University of Minnesota, Personal Communication) 1971.
31. DOLL, J. P. (Unpublished Information) 1972.
32. POLLARD, M., and TEAH, B. A. Spontaneous tumors in germfree rats. J. Nat. Cancer Inst. 31(2):457, 1963.
33. POLLARD, M., and KAJIMA, M. Lesions in aged germfree Wistar rats. Am. Jr. Path. 61:25–32, 1970.
34. POLLARD, M. Spontaneous and induced neoplasms in germfree rats. In: *Environment and Cancer.* Proc. 24th M.D. Anderson Symposium, Houston, 1972. (In press)

35. Berg, B. N. Longevity studies in rats. II. Pathology of aging rats. In: *Pathology of Laboratory Rats and Mice.* Ed. E. Cotchin and F.J.C. Roe, Oxford, Blackwell, pp. 749–785, 1967.
36. Simms, H. S. Longevity studies in rats. I. Relation between life span and age of onset of specific lesions. In: *Pathology of Laboratory Rats and Mice.* Ed. E. Cotchin and F.J.C. Roe, Oxford, Blackwell, pp. 733–746, 1967.
37. Reddy, B. S., and Pollard, M. Effect of germfree status on hepatic xanthine oxidase activity and on bone mineral composition during development and senescence in rats. Jr. Nutrition 102:299–305, 1972.
38. Pollard, M. Carcinogenesis in germfree mice and rats. Proc. 12th Int. Cong. Microbiological Standardization. Karger Press, Basel, 1972. (In press)
39. Sharon, N., and Pollard, M. Effect of cyclophosphamide on lesions induced by persistent LCM virus infection in gnotobiotic mice. Arch. fur die gesamte Virusforschung 34:278–286, 1971.
40. Pollard, M., and Sharon, N. Prevention and treatment of spontaneous leukemia in germfree AKR mice. Proc. Soc. Exp. Biol. and Med. 137:1494–1497, 1971.
41. Coates, M. E. Nutrition and metabolism. In: *The Germfree Animal in Research.* Ed. M.E. Coates, Academic Press, London, pp. 161–179, 1968.
42. Bieri, J. G., McDaniel, E. G., and Rogers, W. E., Jr. Survival of germ-free rats without Vitamin A. Science 163(3867):574, 1969.
43. Pollard, M., and Wagner, M. (Unpublished information) 1971.
44. Orland, F. J., Blayney, J. P., Harrison, R. W., Reyniers, J. A., Trexler, P. C., Ervin, R. F., Gordon, H. A., and Wagner, M. Experimental caries in germfree rats inoculated with enterococci. J. Am. Dental Assoc. 50:259, 1955.
45. Kajima, M., and Pollard, M. Virus-like particles in 3-methylcholanthrene-induced primary and transplanted sarcomas of germ-free rodents. Cancer Res. 27:980, 1967.
46. Gordon, H. A., Bruckner-Kardoss, E., and Wostmann, B. S. Aging in germ-free mice: Life tables and lesions observed at natural death. J. Geront. 21:380–387, 1966.
47. Pollard, M. Senescence in germfree rats. Gerontologia, 17:333–338, 1971.
48. Wilson, R., Sjodin, K., and Bealmear, M. The absence of wasting in thymectomized germfree (Axenic) mice. Proc. Soc. Exp. Biol. and Med. 117:237–239, 1964.
49. Wilson, R., and Bealmear, P. M. Radiation pathology in germfree mice and bone marrow transplantation in irradiated germfree mice. Proc. Intern. Conf. Rad. Biol. and Cancer, Kyoto, p. 107, 1966.
50. von Bekkum, D. W., Radiation biology. In: *The Germfree Animal in Research.* Ed. M.E. Coates, Academic Press, London, pp. 237–267, 1968.
51. Jones, J. M., Wilson, R., and Bealmear, P. M. Mortality and gross pathology of secondary disease in germfree mouse radiation chimeras. Radiation Res. 45:577–588, 1971.
52. Pollard, M., Matsuzawa, T., and Salomon, J. C. Induction of neoplasms in germfree rodents by 3-methylcholanthrene. J. Nat. Cancer. Inst. 33(1):93–99, 1964.
53. Pollard, M. Chemical induction of mammary cancer in germfree rats. Nature 200(4913):1289–1291, 1963.
54. Pollard, M., and Kajima, M. Leukemia induced by 7,12-dimethylbenz(a)-anthracene in germfree rats. J. Nat. Cancer Inst. 39:135–141, 1967.
55. Pollard, M., and Sharon, N. Irradiation-induced lesions in germfree rats. Jr. Nat. Cancer Inst. 47:229–234, 1971.
56. Pollard, M., Kajima, M., and Zacharia, T. P. Rous sarcoma virus in germfree rats. In: *Germfree Biology: Experimental and Clinical Aspects.* Eds. E. A. Mirand and N. Back, Plenum Press, New York, p. 149, 1969.
57. Pollard, M., and Kajima, M. Leukemia in germfree rats. Proc. Soc. Exp. Biol. Med. 121:585–589, 1966.
58. Pollard, M. (Unpublished information) 1971.

DISCUSSION

Stevan Sibinovic, Bionetics Research Lab.: I am interested in the question of mice with neoplasm development. More specifically, when you say that all three strains of mice actually develop neoplasms.

Dr. Pollard: They all have leukemia virus, but only one strain develops it spontaneously. The others have to be irradiated in order to elicit the disease.

Dr. Sibinovic: On B strain, if you use chronic immunosuppressant, do you use it prior to development of the disease or do you stop the disease somewhere else?

Dr. Pollard: We have two papers published on this, one of them in the *Journal of the National Cancer Institute,* and the other in the *Proceedings of the Society for Experimental Biology and Medicine.*

If we treated the mice that were sick with subtoxic doses of cyclophosphamide, we would prolong their life expectancy at least eight to nine times. And if we treated mice before they developed symptoms of the disease, we lost none of them; all of them surviving for thirteen

months. Others that we permitted to live eventually came down with the disease in the course of the next two or three months—no cure.

DR. SIBINOVIC: Do you think that, if you treat animals with the immunosuppressant, you suppress the antigen system and thus retard the development of neoplasm, if, as you mentioned, you didn't before they developed.

DR. POLLARD: Well, what we're actually doing with the immunosuppressant drugs that we have used is keeping a critical mass of lymphoid tissues down. We are really not curing them. All we are doing is destroying a sufficient number of sensitive cells so that there is not this accumulation to produce disease. Now, we have given the following regime to the mice. We have given LCM mice (the Haas strain mice), for instance, five doses of cyclophosphamide at an interval of three months and another five doses every day at an interval of three months. And we have a high incidence of reticulum cell sarcomas among them.

RALPH A. REDDING, Brown University: Do you have any information about an intermediate kind of germ-free animal, the Spartar disease-free rat, in terms of their longevity and kinds of general diseases?

DR. POLLARD: No I don't. As a favor, we have taken some rats with very extensive pneumonitis and run them through the germ-free system, then gave those animals a defined flora and they were free of respiratory disease, so long as they are maintained behind the barrier. In our laboratory, animals are either germ-free or dirty. We don't have any intermediates. It is expensive to keep germ-free animals.

RESISTANCE OF SWINE TO BORDETELLA RHINITIS

W. P. Switzer*

Knowledge of the mechanisms of resistance to bacterial and mycoplasmal infections of the respiratory tract of man and animals has developed more slowly than our knowledge of respiratory resistance to viral infections. This is probably due to our lack of understanding of the processes by which these larger, extracellular microorganisms attach to the epithelial surfaces and resist removal by mucociliary action and to our lack of information about the ability of substances produced by the host to prevent this initial colonization.

INTRODUCTION

Swine are an excellent model for the study of respiratory tract resistance to bacterial and mycoplasmal diseases. Both classes of organisms produce economically significant, widespread, chronic respiratory infections in swine.[16] In spite of this, it has been demonstrated that at least in the case of one chronic bacterial infection, the respiratory surface is capable of developing a remarkable resistance to reinfection.[6]

A discussion of this specific bacterial infection, i.e. bordetella rhinitis will illustrate some of the aspects of this experimental model.

DISCUSSION

Bordetella rhinitis of swine is a chronic disease due to infection of the respiratory system with *Bordetella bronchiseptica*. The infecting bacterium is not exclusively a swine strain since isolates from several species of animals have been demonstrated to infect swine.[14] On initial infection the organism may colonize any level of the respiratory tract but it is most likely to colonize the tracheal and nasal epithelium.[6] It remains localized primarily on the epithelial surface and does not usually invade the tissue. Even though it is localized on the surface, it exerts an effect on deeped tissues, especially the osteocytes and osteoblasts of the nasal turbinate bones. This is manifested as a progressive resorption and hypoplasia (atrophic rhinitis). There is also a fibroplastic reaction in the submucosa and a metaplasia and hyperplasia of the epithelium. These lesions are believed to be the result of a diffusable endotoxin-like material produced by the microcolonies localized on the surface.[1] The specific action of this diffusable product on the osteoblasts and osteocytes may be similar to its demonstrated ability to impair mitochrondial membrane function.[11,10,4,5]

Recovery of infected swine usually proceeds so that the lung and tracheal infection is eliminated before the nasal infection is cleared.[6] It has been suggested that the ethmoid crypts are among the last areas of the nasal cavity to eliminate this infection.[13] Since the organisms are localized primarily on the epithelial surface and since the nasal cavity is accessible to nasal sample collection, the infection can be followed by collection and culturing of nasal samples.[15]

The natural history of this disease in a group of comparably aged pigs housed together gives an insight into the spread/and recovery from this infection as determined by recovery of the organisms from nasal swabs. The anticipated rate of recovery is given in Figure 1. The actual recovery observed in a group of naturally infected pigs is given in Figure 2.[3] Study of these figures reveal that some of the baby pigs become infected within the first week of life by exposure to chronically infected dams. These young pigs serve as good aerosol transmitters and establish an aerosol epizootic within the group of closely housed young pigs. For this reason, naturally infected pigs sampled at 4 to 10 weeks of age have the highest percent of infected pigs as well as the greatest numbers of *B. bronchiseptica* present.

* Veterinary Medical Research Institute, Iowa State University, Ames, Iowa 50010.

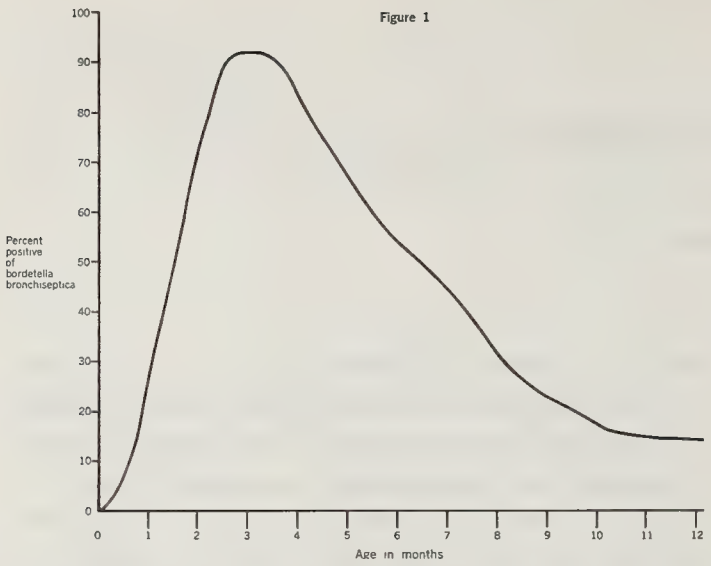


FIGURE 1.—Predicted Incidence of Recovery of *Bordetella bronchiseptica* from the Nasal Cavity of a Group of Naturally Infected Pigs.

After the peak incidence of infection is reached there is a gradual recovery from infection. This is well illustrated in the previous two figures. Unfortunately, recovery in a population is not complete.

An expected 10 to 15 percent of the breeding animals remain infected to initiate the infection in their offspring thus starting the cycle over.[17] It is also apparent from the fact that the disease reoccurs in farrowing after farrowing, that resistance to infection is not transferred from recovered dams to their offspring via colostrum.

Recovered swine are extremely resistant to reinfection. Almost all of the virulent challenge inoculum will have disappeared from the nasal cavity within 1 hour post-challenge, while viable organisms are readily recoverable from the nasal cavities of susceptible control animals.[8] This resistance will last at least for several months.[6] It is not associated with the presence of demonstrable bactericidal substance,[8] nor is it associated with significant levels of circulating, agglutinating antibody.[6]

Not all isolates of *B. bronchiseptica* are of equal virulence for swine. One canine origin isolate has been demonstrated to be regularly eliminated from the swine nasal cavity by the sixth to seventh week post-inoculation.[14] In an effort to determine the reason for this removal, several aspects of the host-basterium interaction were examined. It was found that the early tissue damage produced by the low virulence strain was fully equal to that produced by a so-called virulent strain.[9] The major difference was that numbers of organisms began to decline before significant gross pathology was produced. In an attempt to explain the more rapid disappearance of the low virulence organism from the nasal epithelium, a comparison of phagocytosis of the low virulence and a fully virulent isolate by swine leukocytes was made. The low virulence strain was ingested by leukocytes in much greater numbers than the so-called virulent isolate.[8] It is speculated that the so-called low virulence isolate failed to produce gross lesions simply because it was more rapidly removed from the nasal cavity by phagocytosis.

A comparable situation is believed to occur in pigs that are immunized with a suitable bacterin preparation of *B. bronchiseptica*. In this case the nasal infection is usually cleared in 6 to 8 weeks. Such bacterin preparations will stimulate significant levels of circulating, agglutinating antibodies[7] even though the naturally occurring infection usually does not produce similar titers until about the 4th to 5th month postinfection.[12] It seems likely that by 4 to 5 months postinfection enough nasal damage has occurred to allow invasion of *B. bronchiseptica* into the deeper tissues which results in stimulation of antibodies.

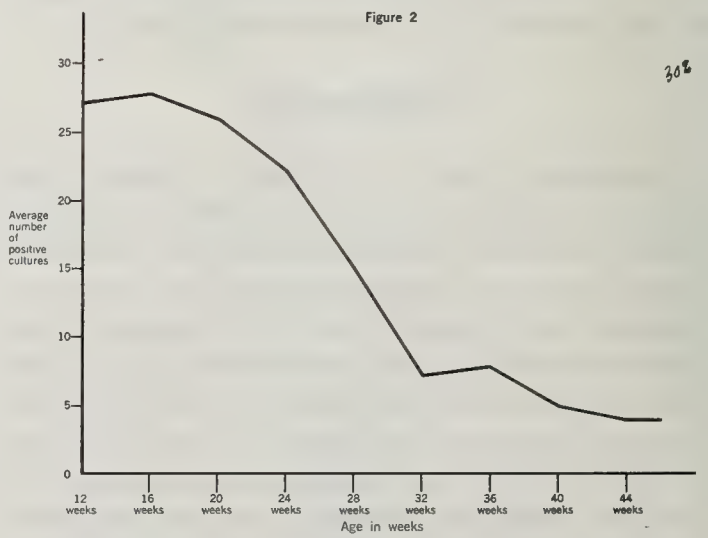


FIGURE 2.—Recovery Rate of *Bordetella bronchiseptica* from the Nasal Cavity of a Group of 30 Naturally Infected Pigs.

Studies are now being undertaken to determine if the stimulation of antibodies by the administration of bacterins of adequate potency will produce rapid and reliable enough clearance of the chronic carrier state to break the generation to generation transmission of this disease. If this proves to be the case then such bacterins will be of great practical value in the control of this disease even though they are not capable of preventing the initial infection. Such bacterins may work through increased efficiency of phagocytic removal of the surface infection.

Consideration of the mechanism of the immediate resistance of the nasal cavity of swine recovered from virulent or low virulence *B. bronchiseptica* infection, either as a result of natural recovery or sulfonamide medication, is more complex. Several facts appear to afford a partial explanation. It has been clearly demonstrated that this bacterium attaches to swine nasal epithelial cells.[8] This is probably the first prerequisite for the successful colonization of a ciliated epithelium. If some provision for anchoring of the bacteria against mechanical removal is not available, then the infectious particle would simply be transported on the muco-ciliary escalator to where it could be swallowed. Epithelial attachment is probably a requirement for any bacteria or mycoplasma to colonize functional ciliated epithelium. It has also been demonstrated that one of the early tissue damages in the area of a micro-colony of *B. bronchiseptica* on ciliated epithelium is the disruption of the cilia.[2] This affords a micro-colony of this organism an additional defense against removal.

Bordetella bronchiseptica also produces a hemagglutinin. There is some preliminary evidence that the epithelial and the red blood cell attaching substance are somewhat similar, if not the same. There is preliminary evidence that nasal secretions collected from resistant swine have some activity in inhibiting bacterial hemagglutination. In order to complete our understanding of this basis for resistance it still must be established that the immediate resistance of the recovered swine nasal cavity is due to a blockage of the epithelial receptor sites on either the bacteria or the epithelial cells by some component in the nasal secretion. Some available information points in this direction. Of importance is the development of a particular antigen settling test (PAST)[7] that is up to 80 times more sensitive in measuring serum and secretion factors stimulated by recovery from this infection. The basis of this test is an altered settling pattern of a standardized bacterial suspension. At the more concentrated levels of serum or nasal secretions this is essentially a microagglutination test. At the higher dilution of test material, the agglutination aspect is left behind and the test is dependent on substances coating the organisms in such a manner that the settling and surface adhesion pattern of the cells is altered. We have demonstrated PAST titers in nasal secretions of resistant, recovered pigs even though no agglutinating antibody titers can be detected. The sera of bacterin injected and chronically infected swine contain both agglutinating and PAST substances.[7] The latter are almost always present at higher titers.

The possible blockage of bacterial attachment sites by factors present in the nasal secretion with subsequent disposal of the bacteria by removal on the muco-ciliary escalator, projects the muco-ciliary surface into a possible role as the first line of immunologic defense against bacterial and mycoplasmal respiratory infections.

SUMMARY

Most attention has been given to the production of circulating antibodies and the passage of certain portions of these antibodies into the respiratory secretions. Secretory antibodies are certainly important defenses but preoccupation with them may cause primary "first line" resistance factors to be overlooked.

REFERENCES

1. Duncan, J. R., Ross, R. F., Switzer, W. P., and Ramsey, F. K. Pathology of Experimental *Bordetella bronchiseptica* Infection in Swine: Atrophic Rhinitis. Am. J. Vet. Res. 27:457–466, 1966b.
2. Duncan, J. R., and Ramsey, F. K. The Structural Changes in the Porcine Nasal Ciliated Epithelial Cell Produced by *Bordetella bronchi septica* Rhinitis. Am. J. Path. 47:601–612, 1965.

3. Farrington, D. O. Personal Communication, Research in Progress.
4. Fetter, A. W., and Capen, C. C. Fine Structure of Bone Cells in the Nasal Turbinates of Pigs with Naturally Occurring Atrophic Rhinitis. Am. J. Path. 62:265–274, 1971.
5. Fetter, A. W., and Capen, C. C. Ultrastructural Evaluation of Bone Cells in Pigs with Experimental Turbinate Osteoporosis (Atrophic Rhinitis). Lab. Invest. 24: 392–403, 1971.
6. Harris, D. L., and Switzer, W. P. Nasal and Tracheal Resistance of Swine Against Reinfection by *Bordetella bronchiseptica*. Am. J. Vet. Res. 30: 1161–1166, 1969.
7. Harris, D. L., and Switzer, W. P. Immunization of Pigs Against *Bordetella bronchiseptica* Infection by Parental Injection. Manuscript in Preparation, 1971.
8. Harris, D. L. Resistance to *Bordetella bronchiseptica* Infection in Swine. Unpublished Ph.D. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology, 1970.
9. Harris, D. L. Personal Communication, Research in Progress.
10. Harris, D. L. and Switzer, W. P. 1971. A Suggested Mechanism for the Pathogenesis of Infectious Atrophic Rhinitis. Can. J. Comp. Med.
11. Harris, R. A., Harris, D. L., and Green, D. E. Effect of Bordetella Endotoxin upon Mitochondrial Respiration and Energized Processes. Archs. Biochem. Biophys. 128:219–230, 1968.
12. Kang, B. K., Koshimizu, K., and Ogata, M. Studies on the Etiology of Infectious Atrophic Rhinitis of Swine. III. Jap. J. Vet. Sci. 33:17–23, 1971.
13. Ogata, M., Koshimizu, K., and Kang, B. K., et al. Studies on the Etiology of Infectious Atrophic Rhinitis of Swine. I. Jap. J. Vet. Sci. 32:185–199, 1970.
14. Ross, R. F., Switzer, W. P., and Duncan, J. R. Comparison of Pathogenicity of Various Isolates of *Bordetella bronchiseptica* in Young Pigs. Can. J. Comp. Med. 31:53–57, 1967.
15. Ross, R. F. Methods of Identification of Certain Etiologic Agents of Atrophic Rhinitis. Proc. U.S. Livestock Sanit. Assoc. 67:512–515, 1963.
16. Switzer, W. P. Bordetellosis and Atrophic Rhinitis. Diseases of Swine. Third Edition, Ed. H. W. Dunne, pp. 617–642., 1970.
17. Switzer, W. P., Spear, M. L., and Farrington, D. O. Swine *Bordetella bronchiseptica* Rhinitis Fact Sheet. II. Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames, Iowa, 50010, 1971.

CHAPTER XII

ANIMAL RESOURCES

Establishment, Organization, Management and Functions of an Investigative and Diagnostic Laboratory within the Department of Experimental Animal Medicine at the University of Washington

Special Considerations in the Use of Calves for Cardiovascular Implant Studies

Improved Resource Utilization Through Animal and Facilities Sharing at the Laboratory for Experimental Medicine and Surgery in Primates

The Role of Veterinary Diagnostic Support Laboratories in a Research Animal Colony

The Research Dog: Random or Colony Reared?

A Support Laboratory for a Medical School Research Facility

Progress in the Development of a Histocompatible Dog

The Primate Information Center as a Research Tool in Studies of the Heart and Lung

ESTABLISHMENT, ORGANIZATION, MANAGEMENT AND FUNCTIONS OF AN INVESTIGATIVE AND DIAGNOSTIC LABORATORY WITHIN THE DEPARTMENT OF EXPERIMENTAL ANIMAL MEDICINE AT THE UNIVERSITY OF WASHINGTON

W. C. Dolowy and L. J. Swango*

The following aspects of the laboratory will be discused in detail: objectives of the laboratory, i.e., balance between diagnostic effort and in depth studies of several conditions; the optimum organization to attain these objectives; raising funds to support the laboratory, sources of funds, state and federal; recruitment of personnel; the optimal space required and attempts to provide adequate space; lists of functions of the pathologist, virologist, histologist, bacteriologist, clinical chemist, secretary; lists of specific determinations performed; relation of the laboratory to institutional committees: relation of the laboratory to the research investigators; relation of the laboratory to the clinical veterinarian; criteria for selecting cases to be studied; how much "interesting outside work" to accept; how and when to refuse additional work; whether to conduct routine pathology on experiments at investigators request; partial vs. complete necropsies; when to conduct virologic determination on the premises and when to subcontract to other agencies; reporting results to researchers and clinical veterinarians; written "vs." or "and" oral reports; data storage and how to submit an annual report.

A retrospective evaluation will be described of the usefulness of the laboratory to the clinical veterinarian and to the research investigators. Also to be described will be such questions as: Who is qualified to judge the usefulness of the laboratory and what criteria may be applicable? What would be done differently if this whole project were to be started anew?

INTRODUCTION**

The laboratory facility which is discussed in this paper was established in 1969 at the University of Washington in Seattle with funds awarded by the Animal Resources Branch, Division of Research Resources, National Institutes of Health. The project was approved as a Diagnostic Laboratory in the Department of Experimental Animal Medicine, School of Medicine, with funding to be made on an annual basis and with competitive renewal of the project to occur at the end of 5 years.

ESTABLISHMENT

Federal funds for support of this project during the first 3 grant years have been awarded in the amounts shown in the following table:

TABLE I.—*Funding of Direct Costs of Diagnostic Laboratory for Grant Years 1, 2, and 3*

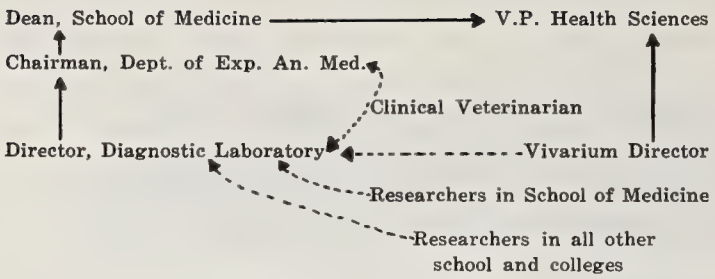
Expense Category	Grant Year 1 (69–70)	2 (70–71)	3 (71–72)
Personnel	35,020	60,750	65,094
Equipment	23,623	4,246	6.640
Supplies	8,557	8,637	10,974
Travel	1,200	1,200	1,200
Publication Costs	1,000	1,000	1,000
Other	600		
	70,000	75,833	84,908

Personnel receiving direct salary support from these funds during grant years 2 and 3 included: veterinary virologist (D.V.M., Ph.D.), virology technician (M.S.), microbiology technician (M.S. equivalent), histology technician (B.S. equivalent), medical technologist (B.S., certified M.T.), secretary, and student helper.

The diagnostic laboratory currently fits into the administrative and service structure of the University of Washington as shown by the following illustration with administrative responsibility shown in solid arrows and those who come to us for service shown in dotted arrows as service input:

* Department of Exeprimental Animal Medicine, School of Medicine, University of Washington, Seattle, Washington.

** Supported in part by funds from grant 5 PO6 RR00489 from the Animal Resources Branch, Division of Research Facilities and Resources, National Institutes of Health.



SERVICE INPUT

The projected goals of the Diagnostic Laboratory were in accord with those of a resource investigative laboratory as defined by McPherson.[1] Since research and in-depth investigations of naturally occurring diseases of laboratory animals are part of the defined mission of an investigative type laboratory, the name 'Diagnostic Laboratory' is somewhat of a misnomer in that it implies solely a service facility. Diagnostic services have certainly been an important function of our facility, but it has been used to a great extent for research and training as well.

ORGANIZATION

The laboratory was organized around key professional personnel in an attempt to maximize the efficiency with which we could carry out the specific objectives discussed below. The director of the facility is a veterinary pathologist (D.V.M., Ph.D.) who is certified by the American College of Veterinary Pathologists (ACVP). In addition, a veterinary virologist (D.V.M., Ph.D.) is on the staff and a clinical veterinarian (D.V.M., M.S.) who is certified by the American College of Laboratory Animal Medicine (ACLAM) devotes about 10 to 15% of his time to the diagnostic laboratory. Since the bulk of our service activities is related to post-mortem examination of laboratory animals or to clinical pathology or bacteriology in support of the clinical veterinarian, he and the pathologist are more directly involved with diagnostic services than the virologist who devotes a greater portion of his time to research. All of these individuals hold faculty appointments in the Department of Experimental Animal Medicine, School of Medicine.

The diagnostic laboratory is divided into sections according to certain disciplines for the purpose of defining the responsibilities of the technical personnel and for division of responsibility among the professional personnel.

A. Histology

The histology section is under the direct supervision of the pathologist. It is staffed by one full-time histopathology technician and one part-time technician or student trainee in histology. The histology laboratory was fully equipped so that almost any type of histopathologic procedure could be performed. A cryostat enables frozen sectioning for histochemical and immunofluorescent procedures. The histologic stains most frequently used are listed in Appendix I.

B. Clinical Pathology

The clinical laboratory section is supervised jointly by the pathologist and the clinical veterinarian. It is staffed by one full-time registered medical technologist (M.T.) certified by the American Society of Clinical Pathologists (ASCP). The laboratory was equipped so that a full complement of procedures could be performed in the areas of hematology, urine analysis, clinical chemistry, and parasite examinations. We do not have the capability for doing correlative determinations of blood pH and blood gases. The procedures used are listed in Appendix II.

Micro methods are used as much as possible and some procedures were performed totally by micro methods. This enabled determinations to be made on individual animals, such as mice, that could not have been done using macro methods. This is especially important in some research studies where statistical analysis are facilitated by individual values rather than a single value on a pooled sample.

C. Bacteriology

The supervisory responsibilities of the bacteriology section are shared by the pathologist and the virologist. Although our intent had been to fill this position with a microbiology technician with a B.S. or M.S. degree, it was staffed by an individual who had a veterinary degree

(D.V.M.) from a foreign veterinary school. The veterinarian employed in this position as a technician has a background in microbiology and, thus was able to meet the needs for a bacteriologist plus contribute considerably more to our laboratory by assisting with necropsies and helping out in the clinical pathology and virology laboratories when needed.

The capabilities in the bacteriology section enable routine isolation and identification of bacteria and testing for antibiotic sensitivity. Identification of bacterial isolates is usually taken to the species level including the gram negative coliforms. Isolates are occasionally sent to outside laboratories for confirmation of identity and/or typing. Examples of isolates which were confirmed in this manner are:

1. *Vibrio parahemolyticus* and *Actinobacillus suis* isolated from a cutaneous abscess on a sand rat imported from the United Arab Republic.
2. *Salmonella typhimurium* isolated from organs taken from a sand rat imported from the United Arab Republic.
3. *Streptococcus fecalis* isolated from tissues taken from a fur seal on the Pribilof Islands.
4. *Pasturella pseudotuberculosis* isolated from tissues taken from a guinea pig.
5. *Listeria monocytogenes* (type 1a) isolated from the uterus of guinea pigs that were aborting near term or giving birth to weakened or dead fetuses.

In addition to the capabilities in bacteriology, some efforts are made to isolate and identify mycoplasma and fungi. *Mycoplasma pulmonis* was isolated on more than one occasion from mice with middle ear infection and pneumonia. The fungi most commonly isolated were *Trichophyton mentagrophytes* and *Microsporum canis*. One fungal isolate from wild-caught, laboratory sparrows was identified as probably belonging to the *Mortierellaceae*.

D. Virology

The virology section is staffed by the veterinary virologist, who has direct responsibility for this section of the diagnostic laboratory, and one full-time virology-tissue culture technician who has an M.S. degree in virology. The program in virology has been limited because of inadequate laboratory facilities. The control of environmental factors is far more critical to the successful operation of a virology-tissue culture laboratory than any other section of the diagnostic laboratory. A laboratory has not been available in which we could control the air supply, "traffic", or maintain "sterile" rooms or cubicles. However, it has been possible to prepare and maintain cell cultures for limited virus isolation and *in vitro* studies. Also, serologic and immunologic studies of a specified nature have been possible.

The cell and tissue culture capabilities have included the use of standard procedures for the preparation and maintenance of both primary cell cultures and established cell lines. Because of the limitations imposed by inadequate laboratory facilities, we have had to limit our studies primarily to canine and feline viruses. Therefore, our emphasis has been on the use of cell culture systems which were susceptible to these viruses. Primary cell cultures prepared in our laboratory have included canine kidney, canine lung, feline kidney, chick embryo, and hamster embryo. In addition to primary cell cultures, a dog kidney cell line and the BSC–1 monkey kidney cell line have been maintained on a continuous basis.

Microtiter techniques have been used as much as possible in serologic tests performed in our laboratory. Viral hemagglutination, hemagglutination inhibition, and complement fixation procedures are routinely done. Other procedures have been used as needed to evaluate immunologic competence, e.g., tests for hemolysin and hemagglutinin antibodies in sera from animals previously inoculated with sheep erythrocytes. Tests for virus neutralizing antibody have been done in both cell cultures and embryonating eggs.

Direct and indirect fluorescent antibody techniques are used for diagnostic and research purposes. For the direct technique, we prepare our own conjugates of fluorescein isothiocyanate labeled globulins using hyperimmune sera from rabbits or goats. Evan's blue dye is routinely used as a counterstain in immunofluorescent procedures. Acridine orange staining of virus

infected cell cultures in Leighton tubes is a common procedure in our *in vitro* research studies.

OBJECTIVES AND FUNCTIONS

The main objectives of our Diagnostic Laboratory in Experimental Animal Medicine have been listed as service, research, and teaching in accord with policies both at the University of Washington and the Animal Resources Branch of the National Institutes of Health. It is difficult to separate these objectives one from the other in terms of independent emphasis. As defined by McPherson,[1] service and research are both emphasized as essential functions of a resource investigative laboratory. Improvement in education, training, and teaching are integrally associated with service and research.

The initial justification for supporting diagnostic laboratory facilities was obviously based on the need for a service to aid in the diagnosis of naturally occurring disease processes in animals used for research. The recognition that to control the diseases encountered would require additional knowledge about the disease processes per se was the justification for supporting research on animal disease with funds awarded to investigative laboratories. This indicates that the logical sequence of events in a resource investigative laboratory such as our diagnostic laboratory should be, first, diagnosis of diseases of laboratory animals, i.e., a service function, and, second, to carry out in-depth studies on the processes of naturally occurring diseases. These in-depth studies should attempt to characterize newly recognized diseases in laboratory animals for the purpose of defining and controlling the disease in the animal or as a potential animal model for studying a similar disease in man, i.e., research. Therefore, the service function of our diagnostic laboratory will be considered first in the following discussion.

A. Service

During the first three years of funding, 1650 accessions were entered into our necropsy records. Each accession consisted of one or more animals and represented only those cases on which gross and microscopic, pathologic examinations were made. In each instance a written report was sent to the investigator describing the findings and pathologic diagnoses. Copies of these reports were sent to the clinical veterinarian, director of the vivarium, and chairman of the Department of Experimental Animal Medicine. The clinical veterinarian and the vivarium personnel are thereby adequately informed and better prepared to take appropriate therapeutic or preventive measures in assisting the investigator with the disease problem. This type of communication is especially important since investigators sometimes come directly to the diagnostic laboratory without first contacting the clinical veterinarian. In addition to the gross and microscopic observations, where possible, bacteriologic and clinicopathologic determinations are made on the same animals to lend support to the morphologic diagnosis. Not included in the 1650 accessions are minor problems which were occasionally presented and on which detailed records were not kept. Minor problems of this type included repeat microscopic examinations for skin mites, simple fractures in small rodents, or paralysis due to fracture or dislocation of the spinal column in rabbits.

In addition to the post-mortem diagnostic services, the bacteriology and clinical pathology sections provide laboratory support for clinical problems encountered by the clinical veterinarian or investigators themselves. The bacteriology section did cultures and antibiotic sensitivity tests on 718 specimens submitted from clinical cases. This represented about 40% of the total service rendered by the bacteriology laboratory. The percentage breakdown of services rendered by the clinical pathology laboratory is as follows:

75%	clinical specimen;
15%	post-mortem specimen;
10%	research specimen.

Service activities rendered by the virology section are again usually in support of the pathologic diagnoses, although some clinical virology and immunology is provided through attempted virus isolations, determination of antibody titers, and by examination of biopsies or tissue scrapings by immunofluorescent procedures. Most of these efforts were limited to

viruses of cats and dogs although a poxvirus was isolated from laboratory sparrows. The sparrow pox diagnosis resulted in a specific research project which is described below under the discussion of the research section.

In addition, the virology section of the diagnostic laboratory has been instrumental in coordinating efforts for the diagnosis of murine viral infections. Sera from several groups of mice were submitted to Microbiological Associates, Inc., Bethesda, Md., for serologic testing. In certain instances, sera is submitted to the National Cancer Institute Ectromelia Diagnostic Laboratory at the New Jersey College of Medicine and Dentistry, Newark, New Jersey, for serologic testing and/or virus isolation studies. As a result of these efforts, it was found that mice supplied to one investigator on an NCI grant had antibody to 6 of the 12 viruses against which serologic tests were run. In another instance, 2 mice from different groups were found to have antibody against mousepox virus (ectromelia). It was also found by virus isolation studies that newly received transplantable tumor material being used in cancer research in mice was infected with mousepox virus. Subsequent to the definitive diagnosis of latent mousepox virus infection in mouse tumor materials, vaccine was obtained, put into use in one colony, and made available to all investigators at the University of Washington who wanted to vaccinate their mice against ectromelia.

Several interesting diagnoses of spontaneous disease in laboratory animals were worthy of publication as case reports. The following are examples of cases that have been reported:

1. Calcinosis circumscripta involving the mammary glands of a New Zealand white rabbit [2]
2. Amyloidosis and dermatomycosis in C57 BL mice [3]
3. Fatal asphyxia in a baboon caused by a laryngeal peduncular fibroma [4]
4. Peripheral carcinoid tumors of the lung in a rhesus monkey [5]

Other diagnoses suggested a need for further research on the disease condition. Some of these have resulted in publishable results as described under the discussion on research.

In order to ascertain the general attitude of investigators toward the service function of our diagnostic laboratory, a survey was conducted among researchers who had submitted animals to this laboratory during the first three years of its operation. A questionnaire and covering letter was mailed to 117 individuals. Of these, 57 (48.7%) returned the completed questionnaire, and 60 (51.3%) did not reply. The following is a copy of the questionnaire with the summary of the 57 replies tabulated:

QUESTIONNAIRE

	Tabulated Summary of Absolute Responses			
	Yes	No	Do Not Know	Quest. Not Ans.
1. Do you recall the fact that an animal was submitted and that you received a verbal or written report of our findings?	54	1	0	2
2. Was our report of any interest to you?	50	4	0	3
3. Did the report help you distinguish between an experimental and spontaneous disease?	30	19	1	7
3.a. Possibly heretofore unknown to you?	11	17	4	25
4. Did the findings ultimately result in more precise diagnosis or treatment by the clinical veterinarian of the condition in this or other animals in your experiments?	24	21	3	9
5. Did the findings ultimately result in changes of purchasing, housing or conditioning procedures implemented by either you or the Vivarium staff	19	28	0	10
6. Assuming that we would be able to continue this service on the same basis, i.e., no cost to you but supported in great part by NIH, would you like to see these services continue?	49	2	3	3
7. Would you like to see additional categories of services added? (If so, please specify: examples would possibly include routine gross and histopathology of tissues from animals on experiment to be examined for result of experimental procedure, rather than for suspected spontaneous disease as is now practiced)	24	9	15	9
8. What other comments do you choose to relate?	21	2	1	33

(Signature) .....

It should be noted that the overwhelming majority of those reporting would like to see this service continued, i.e., 49 positive responses to question #6 and only 2 negative responses. The 2 individuals who responded negatively to that question offered the following comments under question #8:

1. "We have used your services infrequently and therefore cannot justify continued financial support for this operation, regardless of the source."
2. "I decided a few years ago to give up all animal work. The diagnostic service you refer to was peripheral to my major concern. However, it did not achieve a real integration with my interests in the animals. It seemed to be a far more detailed examination than was appropriate in most cases."

In contrast to these negative comments, are the following as examples of comments from researchers who responded positively to question #6:

1. "Rescue of experiments by early diagnosis and treatment of valuable, 2–3 chronic animals is both money-saving and reassuring."
2. "Without your thorough and expeditious promptness, this laboratory could have experienced a disaster."
3. "The service has been most thorough in its workup, and very prompt in giving a final report."
4. "I consider this a most important service; and would further like to state that the quality of this service has been superior."
5. "Your department and the entire vivarium staff have been of tremendous help to me in my research efforts. Many thanks."
6. "Service was prompt. The presence of the diagnostic lab offers a readily available information source much needed in the area."

B. Research

Research activities carried out with the support of the diagnostic laboratory grant are of 2 basic types: 1) investigations to further elucidate the etiology and pathogenesis of naturally occurring diseases of laboratory animals and 2) preliminary studies on certain diseases of animals in an attempt to characterize their potential as animal models for studying an analogous disease in man. Both of these research objectives are in accord with the responsibilities of a resource investigative laboratory as defined by McPherson.[1] Some of our research projects were directed at major problems of laboratory animals that did not necessarily originate through our diagnostic laboratory, e.g., canine viral respiratory disease. In addition, some related research projects were funded by other grant monies although the actual work was carried out within the facilities and by the personnel of the diagnostic laboratory.

Examples of research topics supported in large part by the diagnostic laboratory grant include:

1. Canine adenoviral pneumonia:

   Studies have been carried out on the pathogenesis of pneumonia in young dogs exposed to a canine adenovirus which has been shown to be involved in respiratory disease of dogs in institutional facilities.[6,7] Sero-negative dogs have been exposed by both intratracheal deposition of virus containing fluids and controlled aerosols of the virus. Sequential studies have been done on the development of lesions in correlation with the clinical signs of disease. Histopathologic, virus-isolation, serologic, and immunofluorescent procedures have been used to evaluate the changes. Preliminary results of this study were presented at the 52nd annual meeting of the Conference of Research Workers in Animal Diseases held in Chicago, Illinois, November 28–30, 1971. A manuscript to report the results in detail is in preparation.
2. Comparative pathology and host specificity of a poxvirus isolated from sparrows:

   The pathologic response to experimental infection with a poxvirus isolated from sparrows was studied in 3

species of wild sparrows, canaries, pigeons, chickens, and turkeys. A sequential study was done in sparrows inoculated by various routes in an attempt to elucidate the pathogenesis of the disease. The results have been published.[8] This study was justified on the basis that the initial disease outbreak involved several hundred birds that were part of a large research project that had been ongoing for several years. The acute onset and high mortality resulted in a very costly outbreak. This outbreak was diagnosed by our facility because we had the personnel and technical capabilities of investigating it in detail. We anticipate that our findings and recommendations of quarantine procedures for newly caught birds will aid in preventing another devastating outbreak of this type.

3. Congenital porphyria in cats:

   Congenital porphyria was diagnosed in a female Siamese cat that had died postoperatively. Porphyria was clinically evident in two surviving male offspring, one of which was obtained for clinical studies. The results of preliminary investigations on the genetic, histopathologic, and biochemical aspects of the disease were presented at the 52nd annual meeting of the Conference of Research Workers in Animal Diseases held in Chicago, Illinois, November 28–30, 1971. A manuscript is in preparation reporting the results in greater detail.

   This study was done and is presented here because of the potential value of feline porphyria as an animal model for studying inborn errors of porphyrin metabolism in man. Naturally occurring feline porphyria has only been reported twice previously and it has been characterized in only one genetic stock of cats.[9] This is then an example of the value of a diagnostic laboratory in discovering new animal models for human disease.

In addition to the above, the diagnostic laboratory has provided some support for other research projects which were supported primarily by funds obtained for specific studies by the professional personnel of the diagnostic laboratory. Studies of this type were usually more basic in nature although some were in part applied research. The following are examples of such research topics:

1. The sand rat: An animal model for diabetes mellitus

   A colony of sand rats *(Psammomys obesus)* has been established by several investigators at the University of Washington to study *diabetes mellitus*. Because of the value of the sand rat in comparative medicine, careful attention has been directed toward the study and characterization of naturally-occurring diseases, microbial flora, and normal physiologic parameters. In addition to clinicopathologic and bacteriologic determinations, the diagnostic laboratory has provided some support for histopathologic and histochemical studies for evidence of vascular disease in diabetic animals.

   Naturally-occurring salmonellosis was encountered in one of the sand rats soon after it was imported. There was no evidence of spread of the infection within the colony. *Salmonella typhimurium* was isolated and it has been used to experimentally reproduce the disease in gerbils, which closely resemble sand rats taxonomically and anatomically.

2. Viral-Toxoplasmal interactions in concurrently-infected cells.

   Toxoplasma gondii is not infrequently associated with viral infections, malignant neoplasms, and immunosuppressive therapy. Studies have been supported in part on the interaction between *T. gondii* and virus infection in cell cultures.

3. Oncogenic properties of a canine adenovirus

   Studies on the transformational properties and early oncogenic effects of the

Toronto A26/61 canine adenovirus have been initiated in hamster embryo cell cultures. The eventual purpose of these experiments was to determine whether this virus, which is oncogenic in hamsters, could induce neoplasia in its natural host, the dog, and subsequently, in a pilot experiment in immunologically incompetent subhuman primates.

4. The effect of sulfur dioxide on the susceptibility of mice to influenza virus.

These studies have been done cooperatively with other investigators using specific grant funds as the primary financial support. The diagnostic laboratory provided technical and professional assistance primarily in the area of histology. A manuscript of the results of these studies has been submitted for publication.

C. Teaching and Training

Although the Department of Experimental Animal Medicine does not currently have a formal training program in laboratory animal medicine, formal courses are taught by the departmental faculty for the benefit of graduate students and biomedical scientists in general. Courses offered in this subject area are: Diseases of Laboratory Animals, The Laboratory Mouse, Marine Mammal Medicine, The Laboratory Primate, and Animal Models and Comparative Pathology in Biomedical Research. The diagnostic laboratory has provided resource material and slides for these courses as well as active participation by the professional personnel in classroom lectures.

In addition to teaching formal courses, the day-to-day operation of the diagnostic laboratory provides many occasions for informal education and training. The discussion of postmortem findings with investigators often results in an opportunity to provide new information to the investigator. Consultation has been provided to a number of graduate students relative to animal models and comparative pathology, techniques for using animals in research, and disease problems encountered in their research animals. Some very rewarding experiences have evolved from such contact with graduate students. The following quotation is from the acknowledgments section of one student's thesis:[10]

> "It is impossible to mention all of the involved individuals here, but a few stand out. Dr. Ellis Giddens (our veterinary pathologist) was responsible for making me aware of the ovine disease. His help did not end at this point, however, for he continued to assist me in obtaining the animals needed for the study, autopsying the animals, preparing the material from the animals for study, and giving advice whenever requested. His attitude was epitomized the night he came to the laboratory at one o'clock in the morning to help me autopsy our last sheep so we could get our samples before autolysis set in . . . The staff of the Diagnostic Laboratory of the Department of Experimental Animal Medicine provided technical assistance in the autopsies of the sheep, the preparation of the microscopic sections, and the performance of the clinical chemistry tests".

The appreciation expressed by this individual for services performed by our laboratory in assisting him, emphasizes the contribution we have been able to make to education and training. This example also emphasizes the value to comparative medicine of a facility such as our diagnostic laboratory which is staffed by outstanding professional personnel.

SUMMARY

A retrospective view indicates that during the first three years of existence, our Diagnostic Laboratory had a great deal of success in meeting the objectives for which it was intended. Numerous contributions have been made to the animal health care programs of the Vivarium and University at large. Many research projects have profited considerably from the services we were able to provide. Graduate students have been assisted in research projects because of our input in comparative pathology and biomedical models. With this type of success in performance, we believe we have more than justi-

fied the cost of establishing the diagnostic-investigative animal laboratory.

REFERENCES

1. McPherson, C. Role of the National Institutes of Health in Support of Laboratory Animal Resources. JAVMA, 157:1959–1963, 1970.
2. Henderson, J. D., Jr, and Giddens, W. E., Jr. Calcinosis Circumscripta Involving the Mammary Glands of a New Zealand White Rabbit. JAVMA, 157–663–666, 1970.
3. Henderson, J. D., Jr., and Giddens, W. E., Jr. Amyloidosis and Dermatomycosis in C57 BL Mice. JAVMA, 157:680–684, 1970.
4. Giddens, W. E., Jr, and Dillingham, L. A. Fatal Asphyxiation in a Baboon *(Papio anubis)* Caused by a Laryngeal Peduncular Fibroma. JAVMA, 157:726–729, 1970.
5. Giddens, W. E., Jr, and Dillingham, L. A. Primary Tumors of the Lung in Nonhuman Primates: Literature Review and Report of Peripheral Carcinoid Tumors of the Lung in a Rhesus Monkey *(Macaca mulatta)*. Vet. Path. 8:(In Press) 1971.
6. Binn, L. N., Eddy, G. A., Lazar, E. C., Helms, J., and Murnane, T. Viruses Recovered from Laboratory Dogs with Respiratory Disease. Proc. Soc. Exptl. Biol. & Med. 126:140–145, 1967.
7. Swango, L. J., Wooding, W. L., and Binn, L. N. A Comparison of the Pathogenesis and Antigenicity of Infectious Canine Hepatitis Virus and the A26/61 Virus Strain (Toronto). Am. J. Vet. Res. 156:1687–1696, 1970.
8. Giddens, W. E., Jr, Swango, L. J., Henderson, J. D., Jr, Lewis, R. A., Farner, D. S., Carlos, A., and Dolowy, W. C. Canary Pox in Sparrows and Canaries (Fringillidae) and in Weavers (Ploceidae). Pathology and Host Specificity of the Virus. Vet. Path. 8: In Press, 1971.
9. Glenn, B. L. Feline Porphyria: Comparative Aspects with Porphyria of other Domestic Animals and Man. In *Animal Models for Biomedical Research IV*, National Academy of Sciences, Washington, D.C., pp. 135–148, 1971.
10. Eisele, J. W. A Comparison of Fatty Liver of Pregnancy in Humans and Ketosis in Pregnant Ewes. Thesis for Master of Science degree, University of Washington, 1972.

APPENDIX – I

Histologic Stains Frequently Used in the Histology Laboratory*

H & E—Hematoxylin and Eosin
H.B.F.P.—Hematoxylin-Basic Fuchsin-Picric Acid
Luxol Fast Blue Myelin
P.A.S.—Periodic Acid Schiffs (both standard and picro methyl blue techniques)
Mallorys Phloxine Methylylene Blue
Warthin-Starry Method
Nuclear Fuelgen Stain
Methyl Green
Gram Stain
Mucin Keratin
Giemsa
Mayers Mucicarmine
Congo Red
Oil Red O
Gomori Trichrome
Gomori Methyl Silver
Gomori's Iron Reaction
Glycogen
Weigerts Fibrin
Allochrome Procedure
Prussian Blue
A.F.B.—Acid Fast Bacilli
Lendrum's Stain for Inclusion Bodies
Hematoxylin—Shorr S3 Stain for Inclusion Bodies

* Procedures, indications, and interpretations of these stains can be found in the following references:

Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. Ed. Lee G. Luna, HT(ASCP), McGraw-Hill Book Co., New York, N.Y., 1968.

Thompson, Samuel W., *Selected Histochemical and Histopathological Methods.* Charles C. Thomas, Springfield, Illinois, 1966.

APPENDIX – II

Determinations and Methods Employed in the Clinical Pathology Laboratory Method

Test	Method	Type of Specimen	Minimum amount of specimen to be submitted*
HEMATOLOGY:			
CBC		Whole blood	.5–1 ml
PCV	Micro-hematocrit	Whole blood (EDTA)	.25–.5
WBC	Coulter counter	do	.25–.5
RBC	do	do	.25–.5
Hgb	Cyanmethemoglobin colorimetric	do	.25–.5
ESR	Westergren	do	1–1.5
Reticulocyte count	Brilliant cresyl blue	do	.1
Direct eosinophil count	Pilot's diluting fluid	do	.2
Direct platelet count	Ammonium oxalate	do	.1
MCV		do	Am't. sufficient for RBC, PCV, Hgb
MCHC		do	Do.
MCH		do	Do.
Differential	Wright's stain	do	.1

APPENDIX — II (cont'd)

Test	Method	Type of Specimen	Minimum amount of specimen to be submitted*
Urinalysis		Urine	.5 ml
Ova & Parasites	Direct, sugar flotation, MIF	Feces	Piece size of a pea
Occult blood	Orthotolidine	do	Do.
CHEMISTRY:			
SGOT	Karmen UV (Boehringer Mannheim)	Serum	2 ml clotted blood
SGPT	Wroblewski UV (Boehringer Mannheim)	do	Do.
LDH	Wroblewski-La Due colorimetric (BM)	do	1–1.5 ml clotted blood
Alkaline Phosphatase	Bessey-Lowry colorimetric (BM)	do	1.5–2.0 clotted blood
Total protein	Total solids meter	do	1 ml clotted blood
Albumen	Electrophoresis	do	Do.
Globulin	do	do	Do.
Cholesterol	Liebermann-Watson colorimetric (BM)	do	Do.
Creatinine	Jaffe reaction colorimetric (BM)	do	5 ml clotted blood
Amylase	Smogyi visual (Sigma)	do	Do.
Lipase	CherryCrandall titrimetric (Sigma)	do	2 ml clotted blood
Glucose	Enzamatic with Smogyi filtrate (Glucostat-Worthington) colorimetric	Whole blood,** serum, plasma	1.5–2.0 ml blood .5 whole blood
BUN	Modified Berthelot reaction colorimetric (Hyland)	Whole blood,*** serum, plasma	1 ml cl. blood .25 whole blood
Sodium	Flame photometry	Serum	2 ml cl. blood
Potassium	do	do	Do.
Chloride	Schales and Schales (Sigma) titrimetric	do	1.5–2 ml cl. blood
MICRO METHODS:			
Total protein	Kingsley (Biuret) (Beckman) Gornall, Bradawill, David colorimetric	do	.25–.5 clotted blood
A:G ratio	do	do	Do.
Cholesterol	Zak (Beckman) colorimetric	do	Do.
Glucose	Keston and Teller (Beckman) enzamatic—colorimetric	do	Do.
Phosphorus	Fiske and Subbarrow (Beckman)	do	Do.
SGOT	Karmen (Sigma)	do	Do.
SGPT	do	do	Do.
Bilirubin (total)	Malloy and Evelyn (Beckman) colorimetric	do	Do.
Bilirubin direct	do	do	Do.
Bilirubin indirect	do	do	Do.
Sodium and Potassium	Flame photometry	do	.5–1.5 clotted blood

*Additional sample should be submitted if possible so that tests can be run in duplicate or dilutions made if necessary.

**Suitable anticoagulants—oxalate, EDTA, citrate, heparin.

***Suitable anticoagulants—any common anticoagulant except ammonium oxalate.

Note: Procedures for these methods are listed in text books and manuals of Clinical Pathology and Clinical Biochemistry.

DISCUSSION

F. GELLER, Albert Einstein, New York: Is there a fee for this service?

DR. DOLOWY: No.

C. B. THAYER, University of Iowa, Iowa: Bill, it seems to me that you might well charge a fee for laboratory work done on the planned projects. For biological work, they get money in grants and this sort of thing, but I know it is not enough.

DR. DOLOWY: It is conceivable that you could support one or maybe two technicians on say $15,000 or $20,000 a year this way, but I doubt whether the average investigator would be able to pay even those costs for which we currently receive most of the NIH money. For instance, we're getting $70,000 to $80,000 per year, about 600 reports are going out per year. How much is that? A hundred, a hundred and something dollars per report. So if a man brings in a sick mouse, he's not going to pay $100 for a complete work-up. Even for a dead dog, I doubt whether he'll pay $100 to have a necropsy and bacteriology work-up on a dog. At least not at the University of Washington. They complained about the purchase price of a dog being over $40 or $50 in the first place. So, my guess is that we could not support this elaborate an ef-

fort. You might be able to have, let us say, a hematology and chemistry technician, and possibly a bacteriology technician supported on a recharge basis, but I doubt whether you could go further than that.

S. S. KALTER, Southwest Foundation for Research and Education, San Antonio: You indicate a virological program but you didn't discuss any virological program. Can you elaborate on your virological program?

DR. DOLOWY: A virologist came to us during the second year, and he's been working with us for about one and a half to two years. We first used his efforts primarily for diagnostic work. We've had him attempt to spend most of his time on research, and he, for instance, has worked up the canary pox outbreak in rather detailed studies, which are being published. He's also isolated an adenovirus. We've had respiratory disease problems with newly purchased dogs, and he's isolated an adenovirus from dogs which not only causes a pneumonitis in the dogs but, when transplanted into hamsters, causes tumors which kill the animals. This was not too much of a surprise because human adenoviruses do the same thing. He's now characterizing the pathogenesis of this disease in adult dogs, kittens, and dogs of various ages. He's going to go into primates with it at a subsequent date. He assists in biologic diagnoses by collecting blood from mice and sending it to Microbiological Associates. He also supervises the distribution of material of this sort, but he's primarily conducting investigative work.

J. V. PRINCIOTTO, Georgetown University, Washington, D.C.: Has any thought been given to using this as an adjunct in the training of Ph.D. candidates, rotating them through a clerkship in order to observe their responses to training on this special subfield of veterinary medicine which is concerned with disease and what's being done in a scientific way to prevent problems, or solve problems for animal caretaking?

DR. DOLOWY: We've applied for funds to train veterinarians and also to attempt to give both an MS and a Ph.D. degree in experimental animal medicine. This is the closest that we've come to any graduate programs. We don't have the funds to hire trainees. Ph.D. candidates from other departments do take our courses. For instance, in the primate course we have eleven people enrolled during this particular quarter and so we teach the undergraduate, the advanced undergraduate and graduate students. Technicians, both at the BS level and some of the animal caretakers who do injections in dogs, etc., do attend the lectures but they don't take them for credit.

J. L. WAGNER, University of North Carolina, Chapel Hill: In taking your source material for your pathology, microbiology and biology, do you make any distinction between manipulated animals, animals that have come through research, or those animals that have been maintained for any period of time but have not been manipulated or controlled? Do you make any distinction whether or not the animal has been manipulated and whether or not your pathologists or your laboratory has been expending time and effort in determining why that animal died?

DR. DOLOWY: The distinction that is made is at the time the animal is submitted. The pathologist who at that time was in charge of the laboratory, now the virologist, is the acting director. The director is the one who decides whether he shall accept the animal. He's not obliged to take the animal unless he thinks it's appropriate. Now, his charge is to first look at animals in which there is some reason to believe that a spontaneous disease may have killed them. However, if the researcher is in doubt whether it was a spontaneous disease or experimental manipulation, then the director should also take the animal. This is part of the charge. If it's just a matter of evaluating the animal or a series of animals because it is very likely that there is a deficit of experimental evidence, we're fairly sure occurred due to inadequate experimentation, he will generally refuse them. He'll take the first few just to reassure himself of this fact, of course, but he won't participate as a co-worker in most of these cases. It's just too time consuming.

H. P. SCHNEIDER, Hahnemann Medical College, Philadelphia, Pa.: Dr. Dolowy, it troubles

me in one viewpoint. How do you relate to the Department of Pathology or to the Department of Microbiology? Isn't there a problem here?

DR. DOLOWY: The Department of Pathology appointed our pathologist as a joint faculty member in his department at both my request and the request of the pathologist. This option was also open to the virologist in the microbiology department and he chose not to ask for a joint appointment. He had enough to do with his own research. These options are open whenever we recruit somebody. I leave it up to the encumbent as to whether or not he wants to join another department also. One of the local policies is that all of the funds for a position usually come from the same department, but this is not always the case. In our case, all the funds usually come from our department.

SPECIAL CONSIDERATIONS IN THE USE OF CALVES FOR CARDIOVASCULAR IMPLANT STUDIES

F. T. Galysh and R. F. Wallin*

Calves offer the following advantages as experimental surgical subjects: suitable body size comparable to man, general availability, tractability, and adaptability to laboratory animal quarters. Disadvantages include: rapid growth, thoracic conformation unlike that of man, foaming of blood during extracorporeal oxygenation, and the requirement for special handling and housing facilities. A conditioning program developed in our laboratories includes specifications for weaning, vitamin supplementation, feeding, vaccination and parasite control. The management of the principal diseases of laboratory calves, pneumonia, gastroenteritis and ringworm are detailed. Special handling equipment is described for aseptic collection of donor blood from large calves, for weighing and transport, and for postoperative care of calves. In our laboratories, anesthesia in calves is induced with 5% halothane and maintained with 0.25 to 0.75% halothane and 50% nitrous oxide with oxygen. During extracorporeal circulation succinylcholine and meperidine are used to maintain immobility and analgesia. Lung inflation pressures of 25 to 30 cm H_2O during surgery and the postoperative use of a volume-cycled ventilator delivering 10 to 15 ml/kg tidal volumes have aided in preventing atelectasis. We have carried out over 100 normothermic cardiopulmonary bypass procedures in calves with disc or bubble oxygenators utilizing fresh whole blood and lactated Ringer's solution as priming fluid. Perfusion flows have ranged from 4 to 6 liters per minute and perfusion pressures from 80 to 100 mm Hg. In these procedures blood gas values were as follows: PaO_2, $>$ 100 mm Hg; $PaCO_2$, $<$ 40 mm Hg, and arterial pH, $>$ 7.30. Foaming of calf blood was controlled by adding 2 to 3 grams of Pluronic F-68 to the oxygenator.

INTRODUCTION

Recently a variety of the larger mammalian species has been used in the surgical laboratory for the implantation of cardiovascular devices. In many cases, the calf has evolved as the animal of choice in these studies; and since the calf is a relative newcomer to the laboratory, it has created some new problems. A number of papers at this conference consider in depth the criteria for selection of animal species for particular devices. This paper is based on our experience in the development and evaluation of circulatory assist and related devices and deals with some special considerations in the use of calves as experimental surgical subjects. These include: (1) advantages and disadvantages of the calf for cardiovascular surgery, (2) conditioning of the calf for laboratory use, (3) common laboratory diseases and their control, (4) special techniques and equipment for handling calves, (5) anesthesia and pulmonary ventilation, (6) perfusion techniques and problems, and (7) anatomical considerations.

* Travenol Laboratories, Inc. Morton Grove, Illinois 60053.

THE CALF AS AN EXPERIMENTAL ANIMAL

Advantages

Body size and general availability are probably the main reasons that the calf has become so widely used in recent years in experimental cardiovascular surgery. Under three months of age, the calf is comparable to man in overall body size and is thus more suitable than the dog and other standard laboratory animals for the study of devices and techniques intended for use in man. Because of their importance as farm animals cattle are available in most parts of the country, and calves, especially male animals of the dairy breeds, generally can be economically purchased for laboratory use. Supply and availability are certainly more limited for species such as miniature swine, goats, ponies and even large sheep and dogs. The heart and major vessels of the calf are large and easily exposed and the vessel walls are strong and withstand suturing. Cardiovascular parasites such as the canine heartworm do not occur in this species. Calves

are tractable, easily handled animals which adapt readily to confinement in pens in laboratory animal quarters.

Disadvantages

The immaturity of the calf, and its rapid growth and the associated restrictions on the age and weight range of usefulness in the laboratory probably represent the greatest disadvantages of the boving species. Holstein-Friesian calves are approximately 45 kg at birth and normally gain ¾ kg each day until they weigh about 160 kg. For experimental cardiovascular surgery, calves weighing 65 to 130 kg are commonly used. The importance of the calf's rapid growth is readily apparent; calves cannot be stocked and held for later use since they quickly outgrow useful weight ranges; technical or procedural delays of a week or two sometimes allow calves to grow too large for a study, thus creating additional delays; and in survival studies, calves rapidly outgrow implanted devices, thus limiting the opportunity for long term follow-up.

The apical chest of the calf is grossly unlike the broad, flattened chest of man and can present some unique problems in the surgical installation of bulky devices designed for use in man. Bovine blood readily foams during extracorporeal oxygenation and special precautions must be taken to defoam the blood before recirculation into the body.[1] Ruminant species require special handling to prevent aspiration of regurgitated rumen contents and fatal rumen distention (bloating) during general anesthesia.[2] Methods must be developed for transporting anesthesized and dead calves and disposing of carcasses. Calves require special housing facilities not generally available in laboratory animal quarters; thus renovation or new construction is often necessary before this species can be properly maintained. In spite of the advantages it offers over other species, the calf is obviously not a completely ideal animal for experimental cardiovascular surgery.

Conditioning and Management of Calves

The essentials of calf husbandry apply both on the farm and in the laboratory.[3] Calves for laboratory use should be obtained from reliable sources of healthy animals, preferably commercial breeders with whom a satisfactory conditioning program has been developed. A standing order and a regular delivery schedule for a fixed number of calves help to assure the quality of calves received. Conditioning requirements may vary from one location to another and from one time to the next. The program outlined below has been successfully used by our supplier[A] for over a year and may be modified as necessary. The animals we purchase originate from a large breeding farm where all calves are subjected to this program and those destined for laboratory use are selected by a veterinarian based on uniformity, individual history and physical examinations.

General Specifications

Calves must be of Holstein-Friesian type usually weighing 65 to 130 kg and raised in accordance with a sound herd health program. For a particular series of studies, a 10 kg weight range is specified in advance. Calves may be of either sex but are usually males castrated when 4 to 5 weeks of age. Calves which have received colostrum are taken from the cow at 3 to 7 days and transferred to individual stalls maintained at ambient temperatures which are moderated to 55 to 60°F during winter months. A single intramuscular injection of water-emulsifiable vitamins A, D and E[B] is given at this time. For the first 4 to 5 weeks, calves are bucket-fed increasing quantities of a milk replacer containing 24% protein and 20% fat. Solid food in the form of a commercial calf starter ration[C] is offered after the first week and is gradually increased in quantity until the calf is consuming 750 to 1000 grams daily and can be weaned. Water should be available at all times. We have purposely avoided feeding roughage such as hay which would cause blockage of laboratory drains. A low roughage diet has proven to be satisfactory in calves up to 225 kg.

At 2 to 3 weeks of age, calves are injected with a combination parainfluenza-3 vaccine and

[A] Pel-Freez Bio-Animals, Inc., Rogers, Arkansas 72756
[B] A-D-E 500, Bio-Ceutic Labs, St. Joseph, Missouri 64502
[C] Calf Startena, Ralston Purina Co., St. Louis, Missouri 63199

mixed pasteurella bacterin.[D] At 4 to 5 weeks of age, the above vaccination is repeated and calves receive single injections of (1) mixed bovine bacterin,[E] (2) *Clostridium chauvei-septicum* bacterin,[F] and (3) *Leptospira pomona* bacterin.[F] At 6 to 8 weeks of age calves are treated for external parasites by topical application of Ruelene.[G]

Calves selected for research use are identified 10 to 14 days before shipment and carefully observed for signs of disease. Rectal temperatures are recorded daily. Procaine penicillin G and dihydrostreptomycin are administered by injection prophylactically for 3 days before shipment. At time of shipment, calves must show no clinical evidence of respiratory or gastrointestinal disease and must be accompanied by an official health certificate.

Disease Control

Young calves are susceptible to many diseases and often suffer from several concurrently, but pneumonia and gastroenteritis are the most important. Many calf problems are due to improper feeding, inadequate housing or unsanitary equipment. A suitable feeding program for calves of various sizes should be developed for laboratory use, recorded as a standard procedure and understood by animal care personnel.[5] The importance of thoroughly sanitizing equipment between feedings, properly storing, handling and mixing milk and milk replacers and avoiding overfeeding must be stressed.

The uniform history and age of preconditioned calves is helpful in the differential diagnosis of calfhood diseases. Thus conditions such as omphalophlebitis, neonatal diarrhea, vitamin deficiencies, pediculosis and congenital abnormalities are not usually encountered in the conditioned laboratory animal.

Calf pneumonia may be of bacterial or viral origin but is usually preceded by the stress of chilling or shipping. The disease is contagious and may spread to other susceptible calves; thus the importance of an adequate conditioning program is again apparent. Calves with pneumonia develop high fever (104 to 107°F), rapid respiration and coughing. Diarrhea may precede or accompany pneumonia. The muzzle becomes dry and crusted and generally dehydration ensues.

Calves with pneumonia are treated with sulfonamides (e.g., sulfathiazole, 60 mg/lb/day intravenously) or antibiotics (e.g., oxytetracycline, 5 mg/lb/day intramuscularly) preferably in isolation. In addition, supportive treatment and good nursing care are indicated.

Gastroenteritis is accompanied by diarrhea and most often results from bacterial or viral infections, coccidiosis, sudden changes in diet, or overfeeding. If feeding has been carefully controlled and the dietetic causes of diarrhea can be ruled out, infectious agents are most likely responsible. In this case, calves should be treated with sulfonamides, antibiotics, or nitrofurans along with gastrointestinal protectants, fluid therapy when indicated and careful nursing care. Gastroenteritis in conditioned calves weighing 150 to 300 pounds usually responds well to such treatment.

Ringworm caused by various species of *Trichophyton* is a rather common skin infection of young calves. Lesions are circular, thickened, hairless plaques usually found on the head or neck, about the eyes and in the pelvic region. The disease is a zoonosis and should be promptly identified and treated. Lesions are scrubbed with soapy water to remove scales and fungicides such as 2% tincture of iodine applied topically.

SPECIAL TECHNIQUES AND PROBLEMS

Blood Procurement

Although many investigators collect blood aseptically from adult donor cattle,[6] and others obtain blood from slaughter houses,[7] we have found it convenient to maintain on our premises a donor colony of four large calves weighing from 160 to 275 kg. Donor blood is collected freshly (Figure 1) and aseptically from the jugular vein into ACD or heparin blood bags. These calves donate up to 1500 ml of blood every week or two and maintain hematocrits above

[D] Bar-3, Elanco Products Company, Indianapolis, Indiana 46202
[E] Fromm Labs., Grafton, Wisconsin 53023
[F] Bio-Ceutic Labs., St. Joseph, Missouri 64502
[G] Pitman-Moore Company, Ft. Washington, Pennsylvania 19304



FIGURE 1.—Method of collecting fresh donor blood from large calves.

30% with a supplemental iron-dextran preparation[H] injected intramuscularly after each collection. We have not found it necessary to cross-match blood before use.

We feel that the high incidence of bacterial infection reported in calves undergoing experimental surgery may be attributed in part to contamination of blood obtained from abattoirs. The maintenance of donor animals provides a ready source of sterile blood and eliminates another source of infection.

Handling Equipment

A very useful weighing crate[I] is illustrated in Figure 2. This self-contained scale will accommodate calves weighing up to 300 pounds and serves well also as an animal transport facility.

Calves in which anesthesia has been induced are clipped and scrubbed as they rest on a four-wheeled flat-bed truck and transported from the preparation room to surgery with this device.

During the early postoperative recovery period, calves are kept suspended in a specially constructed canvas sling. This sling, 5½ feet long by 4 feet wide, is fashioned with appropriately situated openings for the legs and for urinary drainage from the male calf. Four reinforced canvas webbings (straps), emanating from each longitudinal side of the sling, are brought over a metal pipe frame. These webbings are attached to winches, which permit



FIGURE 2.—Weighing and transport crate for calves.

[H] Nonemic-100, Armour-Baldwin Laboratories, Omaha, Nebraska
[I] Way-O-Crate, Caswell Manufacturing Company, Cherokee, Iowa 51012

hoisting the animal to a convenient height above the floor. This method of suspending calves has provided a useful and convenient facility for postoperative care and monitoring of this species.

ANESTHESIA IN CALVES

Techniques of anesthesia in the larger mammalian species undergoing thoracic surgery have evolved principally as modifications of procedures used for cardiac surgery in man. Thus, Short et al.,[2] who described in detail their anesthetic technique, induced anesthesia in calves with 4% halothane vaporized into a partial rebreathing circle system with 25 to 50% nitrous oxide in oxygen at a level of 4 liters per minute. This vapor was administered through a mask adapted for the muzzle of these calves in which valve replacement or bypass pump implantation procedures were to be carried out. The trachea was then intubated without recourse to the use of muscle relaxants but with the aid of a specially constructed laryngoscope. Anesthesia was maintained with 0.25 to 0.5% concentrations of halothane until the skin was incised. At this time, nitrous oxide was discontinued; halothane concentration was increased to a mean value of 2% in oxygen until control of respiration was achieved. These calves were then mechanically ventilated with a Bird[J] respirator connected through a Fluotec[K] halothane vaporizer, using 1.0 to 1.5% halothane in a non-rebreathing system. Respiratory rate varied from 12 to 18 breaths per minute, inspiratory pressure was 20 to 30 cm H_2O with tidal volumes of 1 to 3 liters. Depth of anesthesia during surgery was characterized by lack of respiratory efforts, dilated pupils and absence of movement. During extracorporeal circulation these workers used either succinylcholine (50 to 100 mg) or intermittent intravenous injections of 50 to 100 mg of thiopental to maintain immobility. Halothane was again used intermittently to maintain anesthesia until the pleura were closed and discontinued during closure of the chest wall. Calves awakened promptly at the conclusion of surgery.

Many additional anesthetic techniques have been described for cardiac surgery in calves. Larson et al.[8] induced anesthesia with diethylether and then administered 0.5 to 3.0% halothane with oxygen for maintenance. When they employed thiopental instead of diethylether for induction, prolonged recovery times of 24 hours or more were observed.

Bonchek et al.[6] also used diethylether for induction followed by 0.5 to 1.5% halothane in oxygen for maintenance. Additionally, they administered succinylcholine to control muscle relaxation during surgery. Donawick et al.[1] induced anesthesia with 4% halothane, 71% nitrous oxide and 25% oxygen, using a conical face mask. After tracheal intubation, their calves breathed a 50-50 nitrous oxide-oxygen mixture with 0.5 to 2% halothane. When the thorax was opened, they reduced the halothane concentration by one-half and instituted positive pressure ventilation at a rate of 14 to 18 breaths per minute at a pressure of 25 to 30 cm H_2O. During cardiopulmonary bypass they statically inflated the lungs to 25 cm H_2O with the 50-50 nitrous oxide-oxygen mixture.

We have drawn upon the experience of several investigators in developing the following anesthetic technique which we have used with satisfactory results in over 100 calves. Food and water are withheld for 24 hours and calves receive no preanesthetic drugs. Anesthesia is induced with 5% halothane delivered from an anesthesia machine,[L] to a specially constructed face mask, similar to that described by Donawick, et al.[1] Alternatively, anesthesia is induced with the aid of a special face mask containing gauze sponges saturated with halothane. Anesthesia is maintained with 0.5 to 1.0% halothane during skin preparation. The animal is then placed on the operating table and the trachea intubated with the aid of a laryngoscope and a metal obturator placed in the lumen of the endotracheal tube. The endotracheal tube is connected to the yoke of the anesthesia machine. A Bird respirator[M] is used to drive a Bird

[J] Bird Mark 7, Bird Corporation, Palm Springs, California 92262

[K] Fluotec, Harris Calorific Company, Medical Division, Cleveland, Ohio

[L] Ohio Heidbrink, Ohio Medical Products, Madison, Wisconsin 53701

[M] Bird Mark 8 or 10, Bird Corporation, Palm Springs, California 92262

Pneumobag[M] connected to a partially open rebreathing circle with a soda lime cannister. The Pneumobag permits positive pressure ventilation adjusted to an inflation pressure of 25 to 30 cm H_2O at a frequency of 15 to 20 per minute. The anesthesia machine is adjusted to deliver 0.25 to 0.75% halothane and 50% nitrous oxide using oxygen as diluent. Atropine is given intravenously in a dose of 0.01 mg/kg and meperidine hydrochloride is administered intramuscularly in a dose of 1 mg/kg. An indwelling catheter in the jugular vein is connected to an infusion bottle containing 200 mg succinylcholine and 50 mg meperidine hydrochloride in 250 ml of 5% dextrose in water. Generally, 250 to 500 ml are used to maintain immobility and analgesia during extracorporeal circulation and cardiac surgery.

The lungs are statically inflated to a pressure of 25 to 30 cm H_2O during the period of bypass and are reinflated every 20 to 30 minutes during bypass. When extracorporeal circulation is concluded, the animal is returned to the inhalational anesthetic regimen detailed above. The nitrous oxide-oxygen-halothane mixture is continued until the pleura are closed. During closure of the chest wall the animal is allowed to breathe only 50% nitrous oxide-50% oxygen. Within 15 to 20 minutes of completion of surgery, ventilation is carried out with 100% oxygen with an Ohio Medical volume cycled ventilator[N] with the following control settings: tidal volume—10 to 15 ml/kg body weight; rate —15 to 20 cycles per minute; deep breath—50 to 100% of tidal volume added to the tidal volume every two minutes. This ventilator also ultrasonically humidifies its output, thus preventing drying of the airway.

Only two animals have died as a direct consequence of anesthesia in over 100 anesthetic procedures carried out with variants of the above described technique. Both of the animals developed irreversible ventricular fibrillation. Induction time is usually five minutes or less. When anesthesia becomes stabilized, blood pressure is always above 110/70 with occasional values exhibiting elevation to hypertensive levels of 180/130 or greater. Arterial blood gases have ranged as follows: PaO_2, >100 mm Hg; $PaCO_2$, 35 to 40 mm Hg; and pH 7.35 to 7.45 Bradycardia with heart rate falling to 50 beats per minute was observed in a few calves. Hypotension was not a problem so long as halothane concentration was kept below 1.0%.

[N] Ohio 560 respirator, Ohio Medical Products, Madison, Wisconsin 53701

PULMONARY VENTILATION

In our early studies a Bird Mark 8 or Mark 10 pressure cycled respirator was used to ventilate the lung of calves which weighed from 70 to 84 kg. Positive pressure ventilation was carried out at a cycling rate of 15 per minute with the pressure adjusted to 20 cm H_2O. At this juncture, we took note of the work of McLaughlin and associates,[9] who compared pulmonary anatomy in calves, cows, sheep, pigs and other animals and indicated that calves had well-developed secondary lobules with marked interlobular supporting tissue and thick pleura. During thoracotomy, the thick pleura and extensive supportive tissue created a resistance to expansion greater than that in lungs without these characteristics, such as in lungs of man and dogs. Thus, we assumed that higher airway pressures would be required to obtain a similar degree of pulmonary gas exchange in calves than in dogs or man. In our subsequent experiments in larger calves, an Ohio Medical 560 volume-cycled respirator was used. Tidal volume was adjusted so that inflation pressure approximated 25 to 30 cm H_2O to achieve more complete lung expansion. The larger tidal volume coupled with the intermittent deep breaths delivered by this ventilator apparently reversed developing atelectasis. Observations at necropsy confirmed the fact that there was less hemorrhagic atelectasis in animals thus ventilated during the postoperative period than in those animals ventilated with the pressure-cycled respirator. Our observations at necropsy confirmed those of Donawick and coworkers,[10] who found that calves ventilated with minute volumes between 30 and 40 liters per minute did not develop pulmonary atelectasis which was observed by them in calves ventilated with smaller minute volumes of 15 to 20 liters per minute.

PERFUSION TECHNIQUES AND PROBLEMS

We have performed cardiopulmonary bypass in our laboratories in more than 100 calves varying in size from 65 to 130 kg for periods ranging from sixty minutes to more than three hours. These studies have in part related to standardizing a bypass technique for the implantation of heart valves and total artificial replacement hearts. With careful attention to detail, cardiopulmonary bypass is now carried out routinely with maintenance of acceptable perfusion flows and pressures, blood pH, PCO_2 and PO_2 values.

The extracorporeal apparatus employed in these operations incorporates a 25 inch Kay-Cross disc oxygenator,[O] a Travenol Miniprime 6LF[P] or a Bentley Q-100[Q] bubble oxygenator and critically occluded double roller pumps. A Sarns arterial line, or more recently, a Travenol venous line heat exchanger[P] effectively maintained normothermia with water bath temperatures of 40 to 42°C in the Sarns normothermia module.[P] Venous return reservoirs, arterial bubble traps and arterial filters were often added to the circuit. The reservoir serves to facilitate storage and rapid administration of excess blood and administration of fluids and the filter to remove fibrin and other debris that might otherwise find itself into the vascular system of the animal.[11]

In some bypass procedures the carotid artery was also perfused separately with an additional pump to simulate those conditions in which prosthetic devices might be sutured to the aortic root. To gain space for such prostheses in the calf, the brachiocephalic trunk and aortic arch may be clamped during bypass. In this circumstance, separate perfusion of the head is mandatory.

In calves above 110 kg, perfusion flows may exceed 6 liters per minute. In these cases we have perfused both femoral arteries utilizing two separate roller pumps. Dual return sites reduce resistance to pumping at such high perfusion flows and thus minimize potential cavitation effects as a source of gas embolization, which has occasionally been observed in the cerebral arteries (Figure 3).

Oxygenator priming requirements varied between 2,500 and 4,000 ml depending upon the equipment and approach used. Our standard priming solution is a mixture of two parts lactated Ringer's solution to one part freshly drawn heparinized whole blood. Earlier use of an acellular priming solution led to poor venous return and generally poor perfusion. A 6% solution of dextran-70 in 0.9% sodium chloride solution used as a component of oxygenator priming solutions, was found to produce unacceptable post-operative bleeding. The priming fluid now employed allows reproducible blood flows in the range of 4–6 liters per minute for periods of two hours or longer. Perfusion pressures are easily maintained in the range of 80–100 mm Hg by addition of blood or by slight occlusion of venous return lines in those cases where flows are adequate.

Donawick[1] has reported some difficulty in maintaining PaO_2 values above 70–80 mm Hg during cardiopulmonary bypass in calves. However, in a series of ten calves having a mean body weight ± S.E. of 132.7 ± 2.1 kg perfused by us with a disc oxygenator at mean perfusion flows ±S.E. of 4.90 ± 0.23 liters/min., PaO_2 ± S.E. was was 115 ± 11.3 mm Hg, $PaCO_2$ ± S.E. was 34.9 + 1.63 mm Hg and pH ± S. E. was 7.301 ± 0.022. These values were deemed to be quite acceptable for the large calves used in

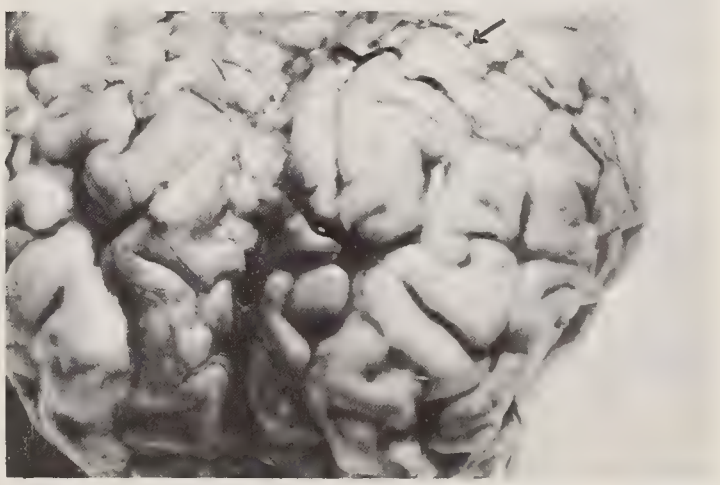


FIGURE 3.—Gas emboli (arrow) in cerebral artery branches of the calf.

[O] Pemco, Incorporated, 5635 Brecksville Road, Cleveland, Ohio 44131

[P] Travenol Laboratories Incorporated, Morton Grove, Illinois 60053

[Q] Bentley Laboratories Incorporated, Santa Ana, California 92707

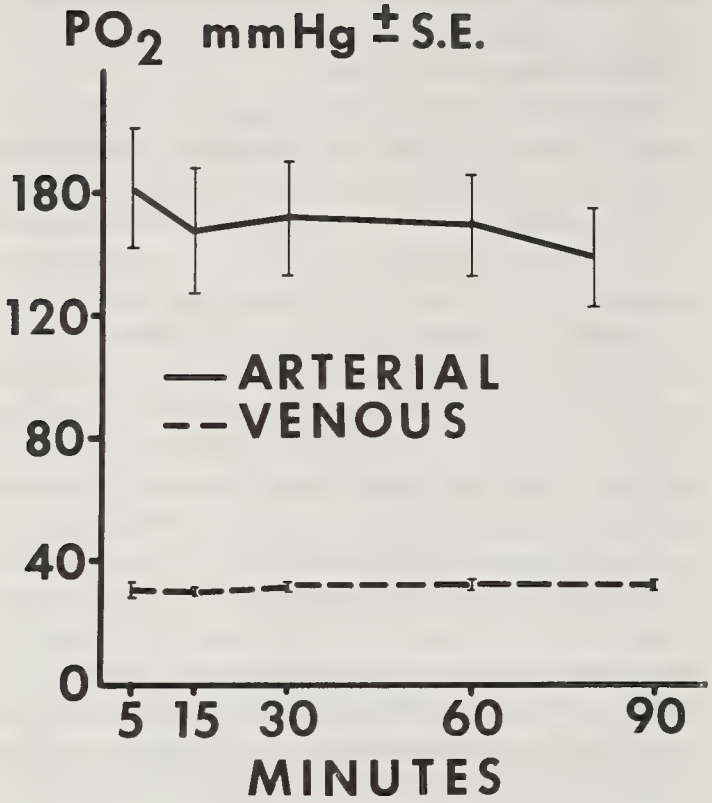


FIGURE 4.—PO_2 values in calves perfused with bubble oxygenator.

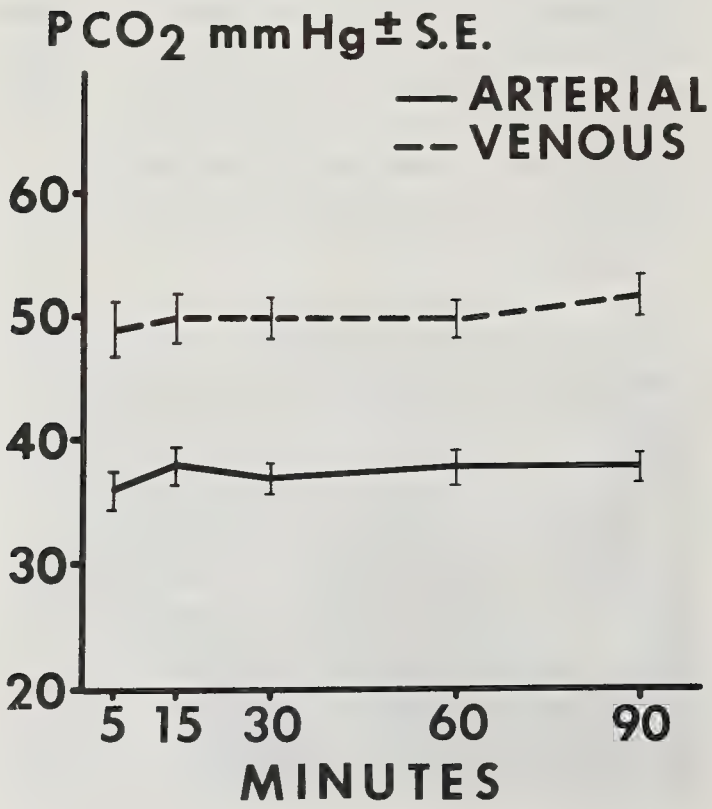


FIGURE 5.—PCO_2 values in calves perfused with bubble oxygenators.

these studies in which the disc speed was 115–120 rpm with oxygen flows of 15 liters per minute.

Figures 4, 5 and 6 depict the mean blood gas and pH values observed in a series of 18 calves having a mean body weight ± S.E. of 113.4 ± 1.7 kg perfused with bubble oxygenators at a mean perfusion flow ± S.E. of 4.78 ± 0.19 liters/min. In this series of studies the oxygen flow to blood flow ratio was generally 2:1. Note that blood gases and pH were within acceptable limits. The Travenol bubble oxygenators were capable of transferring 300 to 350 ml/min of oxygen during these perfusions. Even greater oxygen transfer could be obtained by specially extending by 6 inches the venous oxygenating column of Travenol bubble oxygenators, thereby increasing transit time of blood through thusly modified columns.

Figure 7 illustrates the mean blood gas and

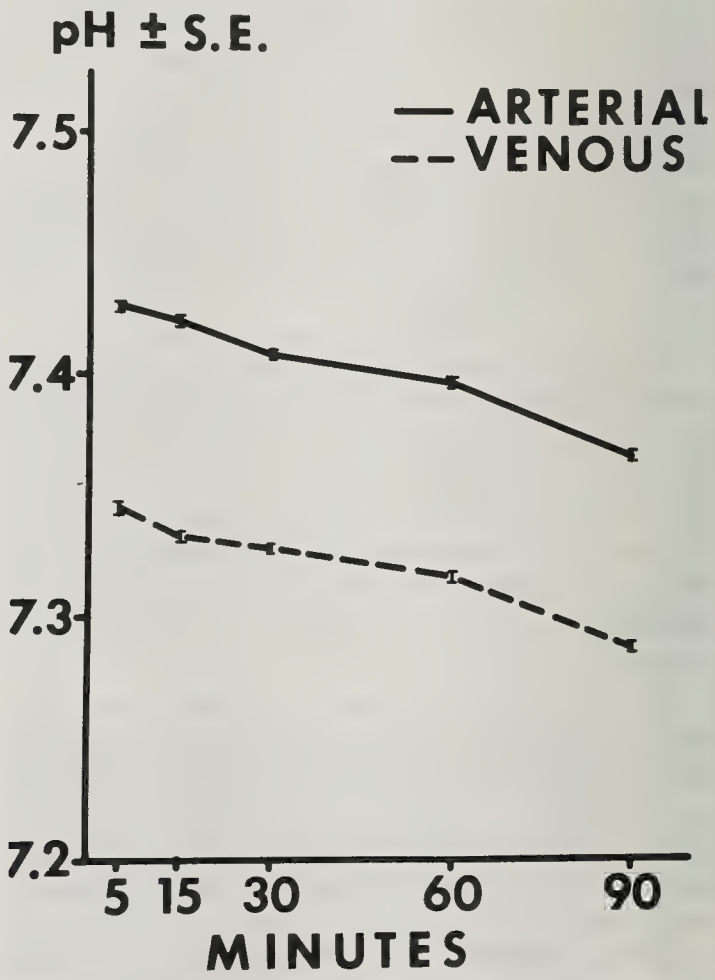


FIGURE 6.—pH values in calves perfused with bubble oxygenators.

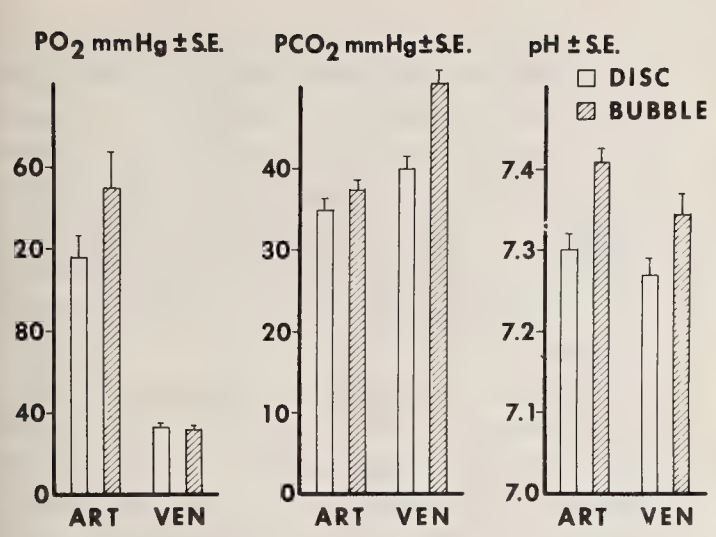


FIGURE 7.—Comparison of mean blood gas and pH values achieved in perfusion with disc (n = 10) and bubble (n = 18) oxygenators.



FIGURE 8.—Inability to effectively defoam calf blood in bubble oxygenator. Note that the undefoamed blood front (white line) had advanced over a considerable portion of the defoaming sponges.

pH values observed in perfusions with disc and bubble oxygenators. These are composite means of all values in all animals taken at 5, 15, 30, 60 and 90 minutes of total cardiopulmonary bypass. Both types of oxygenators were quite efficient in transferring oxygen and carbon dioxide. The apparent lower pH reflecting relative metabolic acidosis in animals perfused with the disc oxygenator is probably related to the greater body mass (weight) of animals in this group.

Foaming of calf blood in the extracorporeal circuit has been a continuous, troublesome problem with both disc and bubble oxygenators. We have not encountered serious foaming with the blood of other species. An adult sized upflow Sarns bubble trap proved to be only partially effective in removing micro-bubbles from the circuit. Other investigators[10] have recognized this problem and combated it by increasing initial heparin dosages to 5 mg/kg and adding Antifoam A or 95% ethyl alcohol directly into the oxygenator. Choosing not to increase heparin dosage and not to use Antifoam A which may embolize systemically, we used ethyl alcohol as a defoaming agent with only modest effectiveness. However, the use of Pluronic F 68,[R] a nonionic detergent has all but eliminated the problems of foaming (Figures 8 and 9). If foaming is a problem as bypass is initiated, from 2 to 3 grams as a 10% solution in water are added to the oxygenator. Pluronic F 68 is well tolerated at this dose level. We have administered as much as 500 mg/kg intravenously to dogs with no evidence of hemodynamic changes or other signs of toxicity.

[R]Wyandotte Chemicals Corp., Wyandotte, Mich. 48192

CARDIAC DIMENSIONS

In the interest of selection of an appropriate animal model for evaluation of the Anstadt-Skinner paraventricular cup (direct mechanical assist device) and as an aid in designing cups for the selected species, we estimated the di-



FIGURE 9.—Effect of pluronic F68 on defoaming of calf blood in a bubble oxygenator. Note that the undefoamed blood front has receded to a point very close to the venous oxygenating column spill-over point onto the defoaming sponges.

mensions of the hearts of three species, these measurements were made in nine calves, two sheep and one pig.

The animals were anesthetized with halothane and the following cardiac dimensions recorded (Table I):

1. Circumference at the base as well as at the largest circumference—by carefully passing a suture around the ventricles and measuring its length.
2. Several diameters with the aid of calipers as follows:
   a. Dorso-Ventral (DV) diameter—from origin of the pulmonary artery on the anterior aspect to the posterior aspect at the base of the heart.
   b. Lateral diameter at base of the ventricles.
   c. Similar DV diameter at the largest diameter of the ventricles.
   d. Lateral diameter taken at the largest diameter of the ventricles.
   e. Oblique diameter I—at right angles to the interventricular septum and at a 45° inclination to both the DV and lateral diameter. This is the larger of the two oblique diameters measured.
   f. Oblique diameter II—parallel to the interventricular septum and at a 45° inclination to both the DV and lateral diameters. This is the smaller of the two oblique diameters measured.
3. Four ventricular lengths were measured, again with the aid of calipers as follows:
   a. Pulmonary artery (PA) to apex.
   b. Coronary sulcus at left atrium (LA) to apex.
   c. Coronary sulcus at right atrium (RA) to apex.
   d. Posterior sulcus to apex.
4. Taper at the ventricular apex was estimated using three rings fashioned of ductile wire. These rings were 10, 15, and 20 cm in circumference. The rings were slipped around the apex and snugged up around the ventricles. The average distance from these rings to the apex was recorded.

TABLE I.—*Dimensions of Cardiac Ventricles of Sheep and Calves in mm.*

Measurement*	Calf 31–0		Calf 32–0		Sheep 1–0		Sheep 2–0	
Body wt., kg	65.9		67.7		73.9		91.4	
Systolic blood press.	105		75		115		160	
Diastolic blood press.	80		45		98		105	
Mean blood press.	88		60		107		125	
	NSR**	VF***	NSR	VF	NSR	VF	NSR	VF
Base circumference	290	335	270	310	280	305	265	357
Largest circumference		370	335	390	317	368	320	382
Base-DV diameter	90	105	103	95	90	90	105	111
Base-Lat. diameter	90	100	103	117	92	100	105	111
Largest diameter-DV	108	105	115	115	100	114	120	129
Largest diameter-Lat.	120	110	115	125	100	114	120	129
Oblique diameter I			130	140		127		
Oblique diameter II			95	105		100		
Length—PA to apex	120	140	120	140	115	130	120	140
Length—LA to apex	110	135	105	110	105	110	115	125
Length—RA to apex	120	135	115	140	110	125	122	130
Length—post. sulcus to apex	95	105	85	100	90	100	100	112
Ring—100 mm	12	18	15	12	17	12	12	16
Ring—150 mm	25	35	30	28	25	25	25	23
Ring—200 mm	40	45	52	43	50	45	52	35–38

* Terms defined in text
** NSR = Normal sinus rhythm
*** VF = After one minute of ventricular fibrillation

The heart was then fibrillated electrically (6.3 volts rms, 60 Hz, applied for 2 to 3 seconds) and allowed to fibrillate for one minute at which time the dimensions already discussed above were estimated. During this procedure mechanical ventilation of the lung was continued. The data are depicted in Table I.

Following these measurements the major cardiac vessels (vena cavae, pulmonary arteries, aorta and pulmonary veins) were ligated and the heart extirpated. The heart was then distended by filling the cardiac chambers with RTV silicone rubber or plaster or saline solution to the approximate size measured previously when the hearts were either in normal sinus rhythm or distended in fibrillation. RTV silicone rubber molds were then cast around these hearts. Positive casts were then made either of plaster of Paris or of Vel-Mix Stone.[8] Shrinkage is minimal with these materials.

Appreciable distention of the fibrillating ventricles is apparent when one compares circumferences and lengths of the ventricles in sheep and calves (Table I). Calves and sheep hearts fibrillating *in situ* are of about the same size. There was poor correlation between ventricular dimensions and body weight (66–91 kg.).

[8] Kerr Manufacturing Co., Division of Sybron Corp., Romulus, Michigan 48174

Hearts, extirpated for a period of time and kept in a frozen state followed by thawing and distention with saline, are about the same size as hearts fibrillated *in situ,* but exhibit some shortening of the ventricles. The distended heart of a large 104 kg. pig was much smaller than distended sheep or calf hearts and was globular in shape. This was particularly evident from the prepared casts of these hearts (Figure 10).

Studies of direct mechanical ventricular assist devices in the rapidly growing calf are complicated by the fixed sizes of such devices and the variable size of the heart. Some means of pre-surgically predicting the size of the heart in this species would be desirable. Since organ sizes generally are related to body weight, heart weight to body weight correlations were done in 68 calves fasted for 18 to 36 hours. Hearts were removed, drained of blood, and weighed. The cardiac weights were plotted versus body weights and a least squares regression line was computed (Figure 11). The correlation coefficient was found to be 0.755 indicating that only a rough correlation exists between heart weight and body weight. The relatively poor correlation was in part related to variability in the volume of ingesta found in the rumens of these calves at necropsy.

Of particular interest in the design of direct mechanical ventricular assist devices (cups, squeezers) is the fact that the length of the posterior aspect of the ventricles in both sheep and calves is much shorter than the length of the anterior aspect. Thus, it may be necessary to design asymmetrical cups unlike those currently being evaluated. Additionally, in distended hearts there is a prominent bulging of the right ventricular outflow tract, which may be traumatized if a symmetrical cup is applied.

DIMENSIONS OF GREAT VESSELS

We sought to compare the dimensions of the aorta in calves and in man since these data have some relevance to the design of intra-aortic balloons and to connectors used with artificial hearts.

With the cooperation of the Department of Radiology at Wesley Memorial Hospital in Chicago, Illinois, thoracic aortograms from 14 clin-



FIGURE 10.—Differences in shape and size of hearts from sheep (left), pig (center), and calf (right).

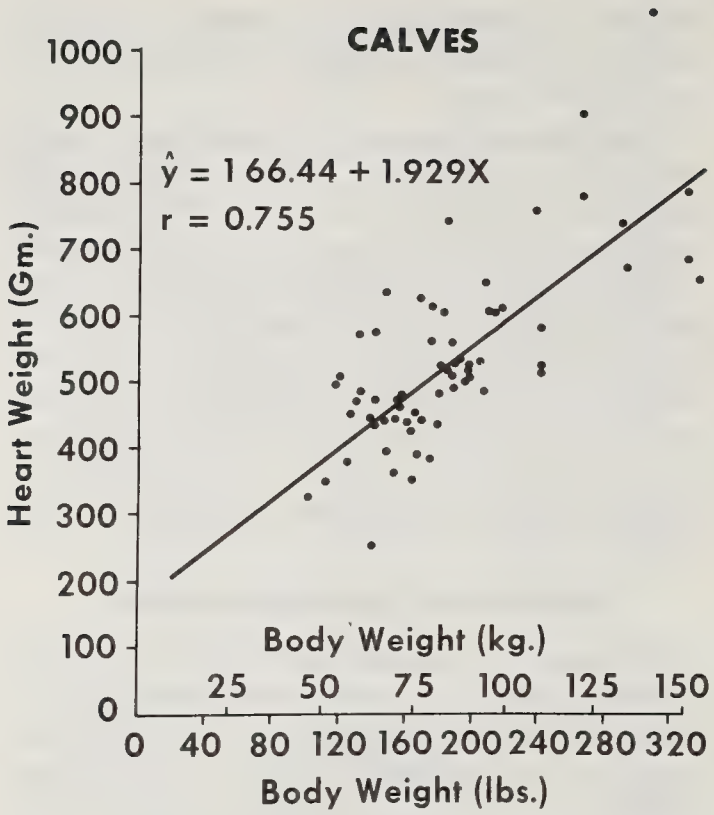


FIGURE 11.—Correlation between heart weight and body weight in calves.

ical patients were examined. The patients' hospital records permitted characterization of sex, height, weight and history. The internal diameter (i.d.) of the thoracic aortas were estimated by direct measurement from aortograms.

Table II summarizes data from the aortograms of the clinical patients. It was possible to obtain the diameter of the thoracic aorta at the level of the diaphragm in only two patients. All measurements in the table are i.d. corrected for film magnification. A normal 1.5 mm wall thickness may be used to calculate outer diameters.

Note that in Patient 6 not much taper of the aorta was apparent from the seventh rib level to the diaphragm. Mean i.d. 1 cm below the aortic arch in females was estimated to be 23.9 mm and in males 28.3 mm. Several factors which may have biased dimensions of these aortas as contrasted to "normal" aortas include long-standing hypertension in a number of these patients and the effect of the high pressure contrast dye injections at the root of the aorta. In considering design criteria for intra-aortic balloons consideration should be given to the fact that aortic i.d. would be reduced in cardiogenic shock states, particularly in those patients with intact elastica.

We measured the aortas of 11 anesthetized calves and found that the calf's thoracic aorta tapers significantly; the i.d. at the level of the ligamentum arteriosum varied from 18 to 25 mm and at the level of the diaphragm from 14 to 16mm. These values tended to be smaller in calves whose blood pressure was reduced below 80 mm Hg by exsanguination. There was poor correlation between aortic diameter and blood pressure or body weight from 50 to 160 kg.

The diameter of the root of the aorta and PA was estimated by actual obturator measurement *in situ* in calves on cardiopulmonary bypass. Similar measurements were made of the cavae (junctional area) and LA (below the annulus).

Structure	*Diameter, mm*
Aortic root	28–32
Pulmonary artery root	27–31
Cavae	18–21
Left Atrium (above pulmonary veins)	42–48

SUMMARY

To aid in the design of cardiovascular devices, several dimensional measurements of the cardiac ventricles and great vessels were made. These revealed that the ventricles of calves and adult sheep are similar in size and taper significantly to an apex, whereas the ventricles of adult pigs are smaller and more rounded in shape. The internal diameters of descending thoracic aortas just below the arch averaged 23.9 mm in human females, 28.3 mm in human males, and ranged from 18 to 25 mm in calves. The internal diameters of the great vessels measured *in situ* in 100 to 120 kg calves were as follows: aortic root, 28–32 mm; pulmonary artery root, 27–31 mm; cavae (at junctional region), 18—21 mm; left atrium (just above pulmonary veins), 42–48 mm.

ACKNOWLEDGMENTS

The authors wish to acknowledge the efforts of Chung-Yuan Lin, M.D., in making the measurements of the cardiac and aortic dimensions

TABLE II.—*Aortic Dimensions in Clinical Patients*

Case No.	Sex	Age	Height (cm.)	Body Weight (kg)	History of Hypertension	History of C.V.A.*	Internal Diameter of Thoracic Aorta 1 cm. below aortic arch	
							R.P.O.** (mm)	L.P.O.*** (mm)
1	F	45	170	79.5	+	+	21.3	21.6
2	F	66	165	66.8	+	+	27.2	27.0
3	F	56	147	63.6	+	+	24.7	25.2
4	F	62	170	68.2	+		23.0	
5	F	51	157	54.1	+	+	17.0	
6	F	31	170	59.1		(1)	19.6 (3)	18.7 (4)
7	F	55			+		22.1	
8	F	53	160	53.6	+	+	27.2	27
9	M	58	178	78.6	+	+	27.3	26.1
10	M	63	168	54.5	+		27.2	
11	M	61	165	75.0	+	+	30.6	30.6
12	M	47	180	77.3	+	+		23.4 (4)
13	M	56				(2)		23.4
14	M	57	170	59.1	+	+	35	31.5

* C.V.A.—Cerebral Vascular Accident
** R.P.O.—Right Posterior Oblique
*** L.P.O.—Left Posterior Oblique
Notes:
(1) Other History—Traum. Rup. of Aorta
(2) Other History—Abscess.
(3) Measured at seventh rib level
(4) Anterior—Posterior just above diaphragm

reported herein. The following additional people also contributed significant technical input: G. McCarthy, A. J. Dauven and B. Abbink.

REFERENCES

1. DONAWICK, W. J., NARA, Y., and BAUE, A. E. Techniques for successful cardiopulmonary bypass in the calf. J. Surg. Res 9:79–86, 1969.
2. SHORT, C. E., KEATS, A. S., LIOTTA, D., and HALL, C. W. Anesthesia for cardiac surgery in calves. Am. J. Vet. Res. 29:2287–2294, 1968.
3. EWBANK, R. The care of the experimental calf. *The UFAW Handbook on the Care and Management of Laboratory Animals*. Williams and Wilkins, Baltimore, pp. 686–695, 1967.
4. MORROW, D. A. Disease control in dairy cattle. In *Bovine Medicine and Surgery*. Amer. Vet. Public., Inc., Wheaton, Ill., pp. 741–766, 1970.
5. AMSTUTZ, H. E. Diseases of Calves. In *Bovine Medicine and Surgery*. Am. Vet. Public., Wheaton, Illinois, pp. 686–710, 1970.
6. BONCHEK, L. I., TATOOLES, C. J., and BRAUNWALD, N. S. Experimental cardiac surgery in the calf. Techniques of anesthesia and operation. Annals of Thor. Surg. 3:211, 1967.
7. NELSON, R. J., MOHRI, H., DILLARD, D. H., and MERENDINO, K. A. Heterotransplantation of the human pulmonic valve to the calf pulmonic area. J. Thor. and Cardiovasc. Surg. 52:863, 1966.
8. LARSON, R. E., MOFFITT, E. A., and MCGOON, D. C. Experimental cardiac surgery in calves I. Anesthesia. J. Surg. Res. 3:101, 1963.
9. MCLAUGHLIN, R. F., TYLER, W. S., and CANADA, R. O., A study of the subgross pulmonary anatomy in various mammals. Am. J. Anatomy 108:149, 1961.
10. DONAWICK, W. J., HIREMATH, I., and BAUE, A. E. Anesthesia, ventilation and experimental thoracotomy in the calf. Am. J. Vet. Res. 30:533, 1969.
11. PATTERSON, R. H., KESSLER, J., and BERGLAND, R. M. A filter to prevent cerebral damage during experimental cardiopulmonary bypass. Surg. Gynec. and Obstet. 132:71, 1970.

DISCUSSION

QUESTIONER (unidentified): What kinds of research and development does your laboratory do?

DR. WALLIN: A variety of research programs and contractual obligations ranging from the development of heart valves and membrane oxygenators to a variety of cardiac assist devices and total heart replacements.

J. R. HELD, NIH: You talked about the disadvantage of the growth of calves which is one that we all recognize, and also you just touched on the subject of sheep. I wonder why you wouldn't consider sheep or goats as perhaps a more appropriate animal because of this.

DR. WALLIN: We've had difficulty, I think as others have expressed, in getting sheep large enough for some of our studies. I think we've found, for many of our studies, animals in the 250 to 300+ range have been most desirable,

and we've just had a very difficult time coming across sheep of that size.

DR. HELD: Why?

DR. WALLIN: Supply has been the problem.

R. B. SHEPARD, University of Alabama, Birmingham: Do you cut through the sternum or travel through one or the other side of the calves?

DR. WALLIN: We've been through both routes.

DR. SHEPARD: Have you got a good way to stabilize the sternum or do you have comments about the sternotomy versus a lateral thoracotomy?

DR. WALLIN: Dr. Galysh who is the co-author on the paper has been intimately involved in the surgical procedures. Perhaps he'd like to respond to that.

F. T. GALYSH, Travenol Laboratories, Illinois: I think stabilization of the sternum has been a real problem in our hands. We don't like to do median sternotomies in calves. The earlier paper has alluded to the infection problem. This has been very real in our hands also in those calves that have undergone median sternotomy.

P. C. SMILEY, Physics International, California: Do you prefer the Holstein?

DR. WALLIN: Again I think it is a matter of availability and a preference in fact over some of the beef breeds which we had experience with earlier. In the midwest, certainly, the Holstein or Holstein type cattle are the most common dairy breeds, and for a variety of reasons, we have avoided the stocky build of the beef calf.

IMPROVED RESOURCE UTILIZATION THROUGH ANIMAL AND FACILITIES SHARING AT THE LABORATORY FOR EXPERIMENTAL MEDICINE AND SURGERY IN PRIMATES

E. I. Goldsmith,* J. Moor-Jankowski** and J. Davis**

The Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) was planned to fill the need for nonhuman primate animals and appropriate related facilities in a large biomedical research community (Greater New York). It was designed to grow stepwise in response to demand from the investigators in the many participating institutions. The validity of the concept has been demonstrated by the continued growth of the laboratory despite the recent general contraction of research activities. During the six years of its existence LEMSIP has assisted 190 scientists. At this writing it accommodates 126 active research programs from 65 institutions. More than 100 shipments of biological materials are sent each month to various investigators. Beginning with 34 primate animals in 1965, the laboratory presently houses 90 apes and 300 monkeys. Floor space has increased from 1600 ft^2 in 1965 to more than 25,288 ft^2 at present. The participating investigators are committed to a program of maximum utilization of animals and facilities, thus achieving remarkable economy in operation.

INTRODUCTION

Despite great scientific and therapeutic advances which resulted directly from the use of nonhuman primates in biomedical research, availability of apes and monkeys for medical experimentation remained severely restricted until recent times. This was due mainly to unavailability of properly conditioned animals and of qualified personnel, lack of knowledge of the animals, lack of methods for their maintenance and handling, lack of proper equipment and, in general, lack of systems for efficient use of primates in medical experimentation. These problems could not be solved in small animal colonies maintained by single investigators for their own studies. Therefore, the protangonists of the use of simians for research purposes realized the need for larger colonies which could develop specialized husbandry and handling techniques, and be used as an animal resource by larger groups of investigators.

The first primate colony for biomedical research was established in 1927 in Sukhumi, Crimea, U.S.S.R. Before W.W.II, the U.S. Government sponsored an experimental monkey colony on an uninhabited island off Puerto Rico. The greatest advances in availability and use of nonhuman primates in research began, however, in the early 1960's with the advent of the N.I.H.-supported Primate Research Centers program. Unfortunately, the geographical distribution of the regional primate research centers made their use inconvenient for New York scientists, although the New York Academy of Medicine estimates that more than 15% of the national health related research is carried out in the New York area.

BACKGROUND

Although the nucleus of the present LEMSIP organization was established by Dr. Moor-Jankowski in 1959 at the Lister Institute for Preventive Medicine, London, and later at the Department of Pathology, Cambridge University, England, the rapid expansion of this primate laboratory came after its location in New York in 1965. In May, 1966, the Committee of Scientists for the Use of Primates in Medical Research was established in New York and became a sponsor and guide of LEMSIP activities. The Laboratory, as it now exists, was developed through imaginative collaboration among investigators from various medical institutions, influential public figures, the Associated Medical Schools of New York and New Jersey,

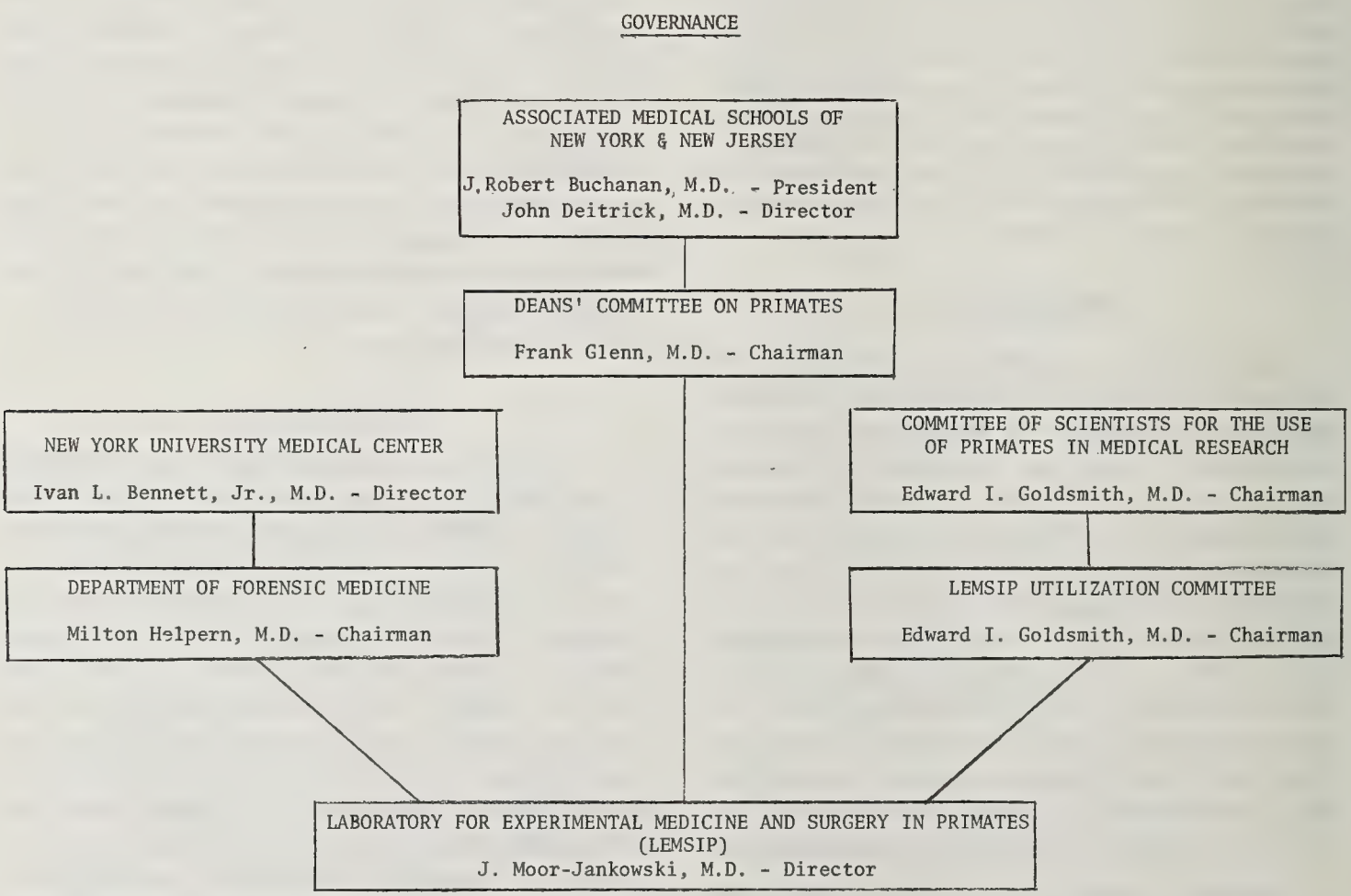
* Department of Surgery, New York Hospital-Cornell Medical Center and the Laboratory for Experimental Medicine and Surgery in Primates of New York University Medical Center.

** The Laboratory for Experimental Medicine and Surgery in Primates of New York University Medical Center.

Federal agencies (N.I.H., N.S.F., U.S.A.F. and U.S.N.), private universities, the City of New York Health Research Council, and foundations (Figure 1) into a smoothly functioning inter-institutional research facility. [1,2]

The physical plant and operations at LEMSIP have been developed with significant departures from pre-existing models of laboratory animal housing. The design was based on a set of requirements formulated as a result of prior experience using primates in biomedical experimentation. They had to be adapted to the specific needs of New York's biomedical research community and to the scarcity of funds for capital investment. The requirements are as follows:

1. Easy accessibility of the animal for handling.
2. Greatest possible visibility of the animal in the cages for purposes of observation.
3. Optimal size of animal rooms, which are self-contained to prevent the spread of possible infections between animal rooms.
4. Maintenance methods that would limit spread of possible infections among animals within the self-contained animal rooms.
5. Variation of housing methods in conformity with the variable behavior and living habits of various primate species, without, however, assigning anthropomorphic traits to primate animals.
6. Flexibility of housing space that would be amenable to low-cost transformations whenever required by experimental protocols.
7. Highest possible efficiency and automation to eliminate routine and repetitious unskilled and semi-skilled tasks in order to



GOVERNANCE

FIGURE 1.—Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP).

keep labor costs down and to attract superior research-oriented personnel.

8. Overall economy in housing, equipment, and operation, by careful analysis of all costs. Emphasis has been placed on achieving maximum research results per dollar of expenditures.

The cage design for all animals, including the 5′ × 5′ × 6½′ chimpanzee cages, is derived from the labor saving cage systems of the poultry industry. All cages are suspended on walls of the animal room; a 150 lb. baboon cage can be easily removed by one technician using LEMSIP's cage lifter; the heavy large chimpanzee cages are easily moved on either overhead trolley tracks or by fork lift cage lifters. LEMSIP's dry system of waste collection consists of plastic sheeting placed under the cages and collected every 24 hours for smokeless incineration.

All animal housing and laboratories consist of modular transportable units designed by the LEMSIP staff; the units are far less expensive then conventional buildings and allow flexibility of housing design.

The modular housing units as well as the caging and other equipment have been purchased for LEMSIP by participating institutions (Figure 2). Several of them have adopted LEMSIP caging for their own intramural animal housing, so that the animals can be easily housed when brought from LEMSIP to the investigator's institutions when required by research protocols. LEMSIP activities are supervised by the Utilization Committee consisting of representatives of users of the Laboratory.

FIGURE 2.—Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP).

Owners of Capital Equipment

January 1972

CITY OF NEW YORK
COLUMBIA COLLEGE OF PHYSICIANS AND SURGEONS
CORNELL UNIVERSITY MEDICAL COLLEGE
GEORGETOWN UNIVERSITY MEDICAL SCHOOL
IRVINGTON HOUSE INSTITUTE OF NEW YORK UNIVERSITY MEDICAL CENTER
LABORATORY FOR EXPERIMENTAL MEDICINE AND SURGERY IN PRIMATES (LEMSIP)
MOUNT SINAI MEDICAL SCHOOL
NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NEW YORK BLOOD CENTER
NEW YORK UNIVERSITY MEDICAL CENTER
UNITED STATES AIR FORCE

A basic objective of our Laboratory consists of helping investigators establish their own research programs at LEMSIP or in their own institutions, or in both. In such a way, the professional know-how of our staff, the animals, and the available facilities are used to support the research of many workers, who in turn provide their own professional abilities to solve the problems of research and animal care that are outside of our own area of competence. The collaboration is not limited to the help of scientists from various institutions in problems arising in our own laboratory; we are also able to arrange exchange of information on capabilities and needs, as well as collaboration between workers from different institutions whose specialties complement one another's areas of research. For instance, help of an obstetrical team from New York has made possible investigation of viral transfer from mother to fetus carried out by a New England team in our laboratory.

Each of the consultants is motivated by his interest in the primate laboratory that supports his own research programs, and by the benefits which he, in turn, derived from the consultations provided by others. Thus, the primate laboratory gains the time, effort, and talents of people who might otherwise not be available for consultations were it not for their scientific motivation.

Many research projects require only periodic supply of a large number of fresh normal samples, as, for example, studies of blood groups, serum specificities, biochemistry of hemoglobins and viral flora. In order to justify better the maintenance of relatively large numbers of animals for any one of such purposes, additional investigations are carried out on the same animals, according to research plans which do not interfere with the normal condition of the simultaneously investigated parameters. Moreover, a large number of animals can accommodate more research projects per animal by allowing for rest or incubation period on one kind of experiment while using the same animals in

different kinds of studies. Furthermore, the larger professional and technical staff, justified by more research studies, helps to solve the problem of maintaining a full 24-hour, seven-day week of maintenance and observation.

An investigator who wishes to avail himself of LEMSIP's assistance has to submit a protocol for evaluation by the LEMSIP Utilization Committee. Prior to submission of a protocol the project is, in general, discussed by the investigator with LEMSIP staff members who may advise on details of feasibility. During the past six years no protocol formally submitted to the Utilization Committee after such conferences has been turned down. Most of the protocols were modified following advice of the Utilization Committee in the preliminary talks. In general, the modifications included selection of more appropriate primate species, avoidance of the use of endangered species, and changes in methodology to make the program compatible with techniques utilized in primate animal experimentation. Protocols have also been generally modified to improve cost effectiveness and to permit animal sharing by other programs.

For the purpose of animal sharing, the animal is, in general, assigned to a principal program which also carries all or the major part of the cost of animal purchase or maintenance. When the Utilization Committee determines that other programs can benefit from the use of that animal, the owner's permission is requested, and if obtained, the animal may be simultaneously assigned to another program.

The course of experimentation is then closely monitored by the Utilization Committee to avoid any possible conflict between programs assigned to the same animal. Due to the understanding and cooperativeness of the community and of close supervision of the programs by the Utilization Committee, such conflicts have been avoided. Depending on program work loads, the secondary programs may contribute toward the cost of animal maintenance. Fees for services rendered to user investigators (research resource in Figure 3) provide one third of LEMSIP's operating income.

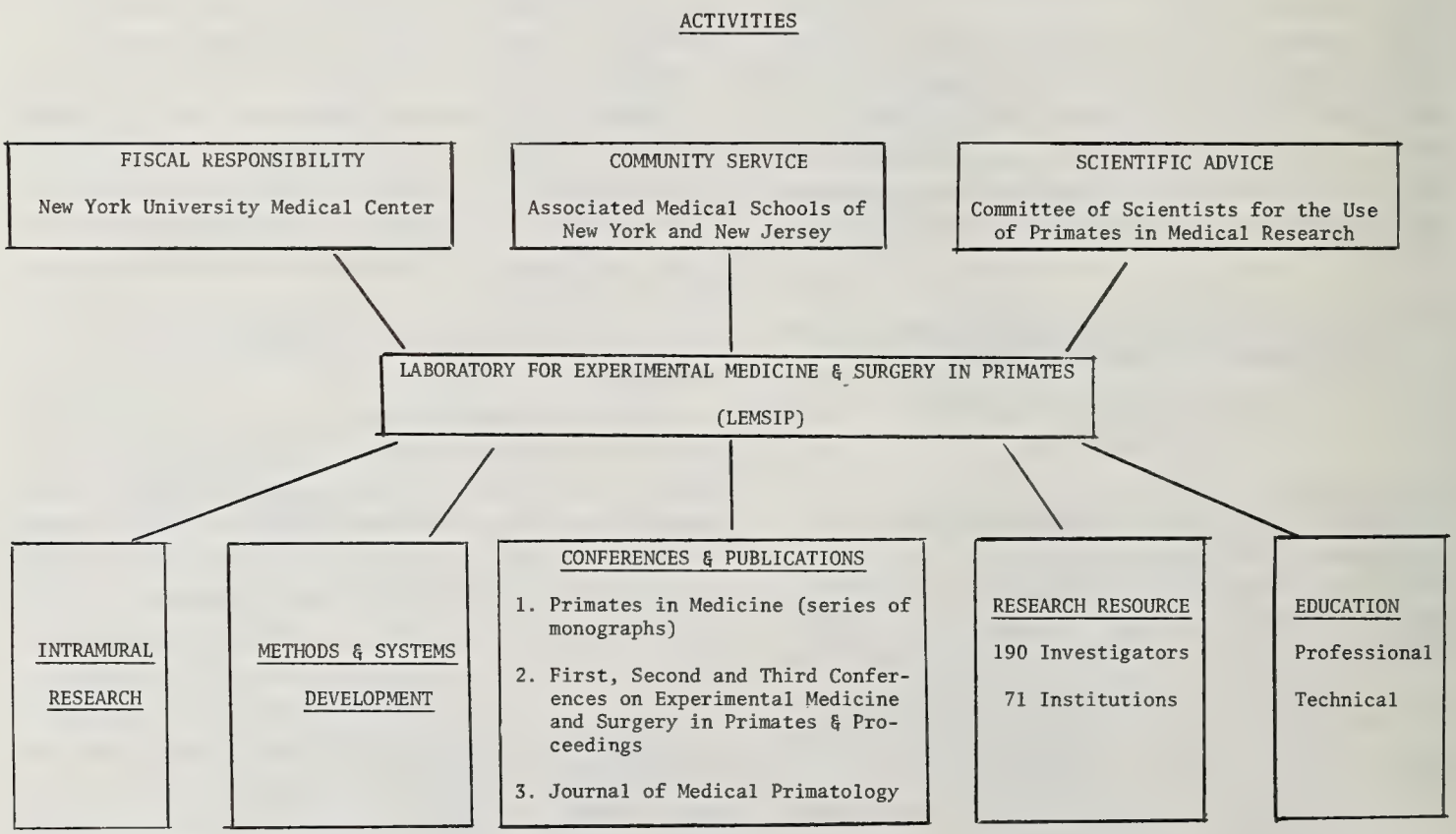
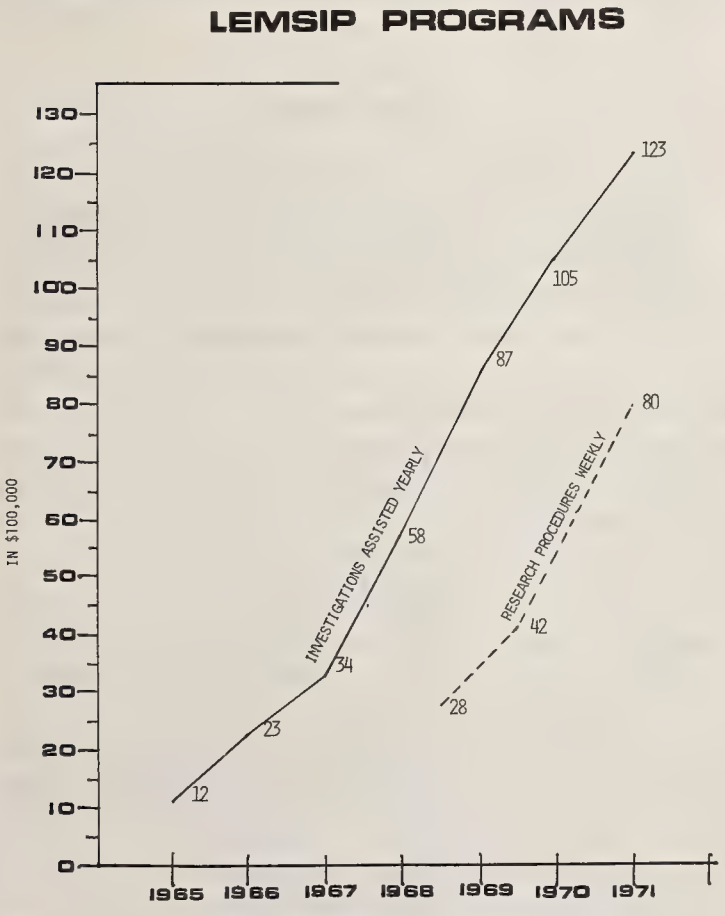


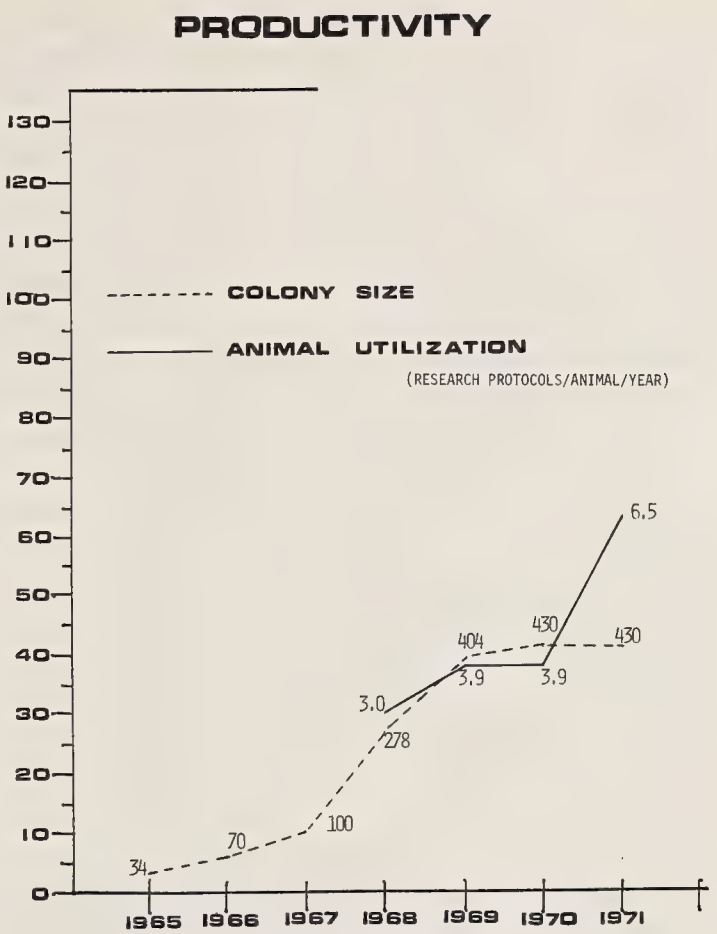
FIGURE 3.—Laboratory for Experimental Medicine & Surgery in Primates (LESIP).

During the fiscal year 9/1/70-8/31/71, the technical support received by LEMSIP investigators doubled as determined by the increase in the number of weekly average research procedures requiring assistance from our technical force (from 42 to 80 a week—Figure 4). This increase demonstrates in large part the increased sharing of animals among investigators, whereas the size and turnover of the LEMSIP animal colony has remained relatively stable (Figure 5). This dramatic increase in work performed upon an animal colony of constant size can be further interpreted as a reflection of increasing productivity. Efforts to encourage animal sharing had previously been successful to the extent that each animal was being used for an average of 3.9 programs per year in 1969 and 1970. However, with the need to minimize expenses created by recent contraction of research budgets, we have seen an even more dramatic rise in utilization to a present level of 6.5 programs per animal per year (Figure 6).



PROGRAMS

FIGURE 4.—Laboratory for Experimental Medicine & Surgery in Primates (LEMSIP).

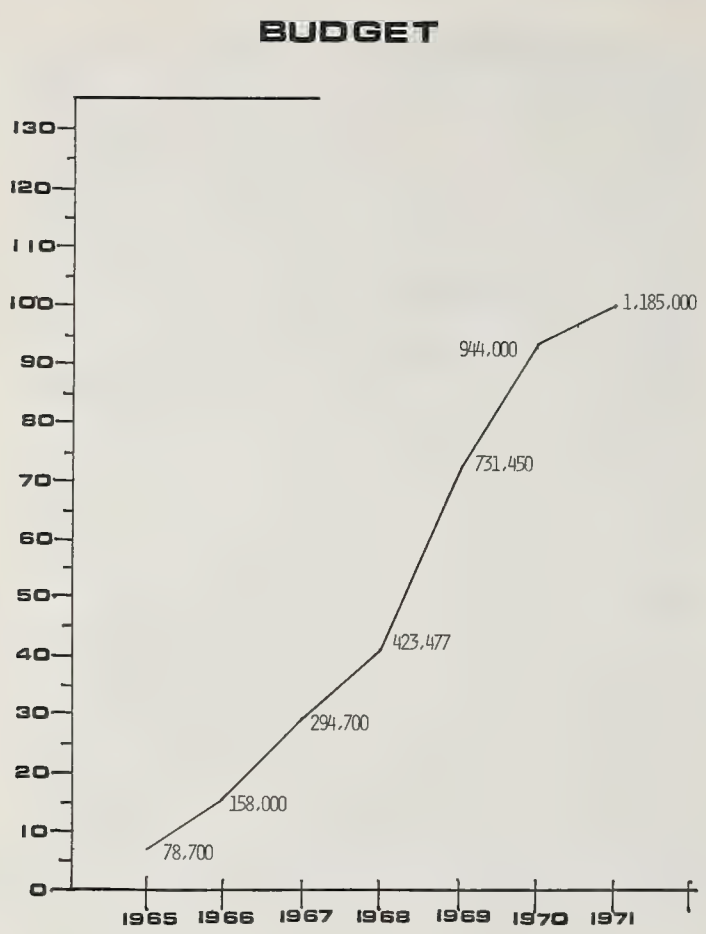


PRODUCTIVITY

FIGURE 5.—Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP).

The concept of conservation of animals for ecologic as well as economic reasons is carefully regarded. Therefore, animals are rarely if ever destroyed except for compelling reasons when scientific information is to be gained by autopsy examination. Otherwise, when a particular study is completed, the animal is reassigned to another investigator and/or program, thus implementing our concept of sequential as well as simultaneous utilization.

Figure 7 outlines an example of compatible multiple utilization of an experimental preparation when care is taken in planning and execution of the protocol to be sure that many investigators benefit. This multiple participation is actively pursued by staff and Utilization Committee members who, knowing of LEMSIP programs are able to attract, advise, and assist other investigators in the fulfillment of their projects. When this concept was first instituted,



BUDGET

FIGURE 6.—Laboratory for Experimental Medicine & Surgery in Primates (LEMSIP).

many observers expressed skepticism concerning its feasibility, but we can now state after six years experience that: (1) productivity is still rising, and (2) there has never been a serious dispute in the execution of the program. We believe that this experience can serve as a prototype for other communities in the future.

SUMMARY

During the period 1969–1970, each animal was used for an average of 3.9 protocols annually. However, with recent budgeting constrictions it was possible to further increase utilization to a present level of 6.5 projects annually. This is achieved by simultaneous as well as sequential use of the animals closely coordinated by an active utilization committee. Another important source of economy and scientific productivity has been the active and generous cooperation and collaboration by participating scientists from different disciplines who have provided advice and expertise that would otherwise be expensive if not unavailable. The Laboratory now functions as a truly interinstitutional program providing a unique resource and a possible prototype for shared facilities elsewhere.

REFERENCES

1. MOOR-JANKOWSKI, J., and GOLDSMITH, E. I. The laboratory for experimental medicine and surgery in primates: Background and policy. In: *Medical Primatology*, S. Karger, Basel/New York, 1971.
2. DAVIS, J. H., and MOOR-JANKOWSKI, J. Laboratory for experimental medicine and surgery in primates

FIGURE 7.—An Example of Simultaneous Sharing of a Baboon Pregnancy

PRIMARY PROGRAM:	— Prevention of premature labor through pregnancy,	and Effects of labor upon the fetus—breeding, maintenance physiological observations and experiments at term.
	Columbia-Presbyterian Medical Center, New York, N.Y.	
ASSOCIATED PROGRAMS:	1. Induction of ovulation—	N.Y.U. Medical Center
	2. Blood grouping of parents and fetus—	N.Y.U. Medical Center
	3. Production of timed pregnancies—	LEMSIP
	4. Maternal urine during and after pregnancy—	Cornell University Medical Coll.
	5. Maternal gallstone formation—	Cornell University Medical Coll.
	6. Maternal urine during pregnancy—	N.I.C.H.D., Bethesda
	7. Amniotic Fluid—	University of California, San Diego
	8. Amniotic Fluid—	McGill University, Montreal, Canada
	9. Amniotic Fluid—	Columbia-Presbyterian Med. Center
	10. Fetal & maternal blood—	Montefiore Hospital, N.Y.
	11. Placenta—	N.I.C.H.D., Bethesda
	12. Neonatal liver—	N.Y.U. Medical Center
	13. Neonatal brain & kidney—	Brain Research Inst., London
	14. Neonatal skin & bone—	Ortho Foundation, Raritan, N.J.
	15. Neonatal milk—	New York Blood Center, N.Y.
	16. Neonatal blood—	LEMSIP

(LEMSIP): design and operation. In: *Medical Primatology*, S. Karger, Basel/New York, 1971.

DISCUSSION

CHAIRMAN MCPHERSON: You said that you went back to the person who had the principal use of the primate and asked his permission to use it on a simultaneous protocol. I was wondering what your experience on acceptance rate of this has been. How often do you get turned down?

DR. GOLDSMITH: Well, I should say that the model for the development of this concept comes from the great generosity of Alexander Weiner, who, working with Dr. Moor-Jankowski, actually was the primary investigator who helped us develop this whole program. It was his baboons, his and Dr. Moor-Jankowski's, which were used by the rest of us in getting this thing off the ground. So with that example, it was very difficult for any subsequent investigators to say "no" to simultaneous and sequential utilization.

I can remember one situation where an investigator was studying alcoholism in relation to liver function and whose program called for sequential liver biopsies over a period of several years. We were anxious to obtain liver tissue to give to other people who were also interested in the same material. He refused. We spoke to him at some length, and he demonstrated to us that the biochemical and histologic studies that he had programmed for his animals simply made it impossible for him to part with any of that liver tissue. He was willing to allow us to use other biologicals from his animals but no liver. The Utilization Committee accepted that refusal. I don't remember any other example. Now I should say that the formal presentation of the request to the investigator usually is preceded by considerable discussion, and so the acceptance rate, as you say, has been extremely high. This has brought together a lot of people who ordinarily don't talk to one another. For example, I previously mentioned about our work in the surgical treatment of schistosomiasis. We have a number of animals harboring schistosomal infections. One of my ophthalmologic friends heard about these baboons and asked if he couldn't do lens transplants. Since there was no incompatibility at all, we were very happy to have him come and operate on the eyes of these animals. So there are many ways that people and projects can be put together.

JULES CASS, Veteran's Administration, Washington, D.C.: Dr. Goldsmith, apparently the system that you're using, accountability for funding, must be an integral part of what you're doing and the success that you've had. It does relate to the fact that you have not compromised the fundamental facilities and the humane aspects of the maintenance of the animal. It would seem that you have a system that is superior to the ones we've been working with and I say "we" in the general scientific community, because we periodically find that we are in need of large sums of money in order to upgrade what we are doing and to avoid the compromises that are so frequent in the animal support area.

DR. GOLDSMITH: Thank you Dr. Cass. I think sometimes there's an advantage in being poor. This has been a bootstrap operation and a lot of what you saw in the way of development of facilities has come from necessity. For example, you notice that all of those cages are galvanized. There isn't a stainless steel cage in the whole place. This has been partially due to the fact that the evolution of caging is something that we're actively pursuing. The cages that were used some time ago have now evolved into better models but we see no reason to invest in what might be called permanent caging during a period when we are learning about how to improve our methods. I should say parenthetically that we don't really have any great problem with the galvanized cages. Now in the case of the chimpanzees, the use of aluminum caging has been a tremendous help and a great saving in money, too.

DR. POWELL, NIH: Apart from the remarkable success of this program in terms of its scientific interests and its example for social cooperation and organization, can you say a word about what must be an interesting logistics problem. How do you handle the administrative and logistics problem of getting the maximum information out of all the experiments and pro-

gramming different people together on the same animal? Do you use, do you depend heavily on computer aids at all?

DR. GOLDSMITH: We have no computer. We have no access to a computer. In reference to the last slide I showed, with the utilization of that pregnancy preparation, the computer in that case was Wendell Niemann's brain. Dr. Neimann is a veterinarian who has been with us for some four years now. He came from NIH and is in charge of the reproductive, perinatal, growth and development programs at the laboratory. He coordinates a large part of this kind of distribution of materials. The various other members of the staff also participate, and a good part of their activities is involved in sending biological materials all over the world.

As far as the cooperative invasive use of animals, this can be done too. For example, when Cesarean sections are performed in the reproduction programs, our surgeons, who are interested in biliary tract disease, are right on the spot to get their bile specimens by aspiration of the gall bladder. So there is an investment on the part of the investigators, too, to come to the laboratory and to participate in some of these shared experiments. I don't know that we would do better if we put this on a computer, but I don't know enough about computers to be able to say. I do know that the essential point here has been the voluntary and eager cooperation by our staff members and participating scientists.

THE ROLE OF VETERINARY DIAGNOSTIC SUPPORT LABORATORIES IN A RESEARCH ANIMAL COLONY

A. M. Jonas*

Increased sophistication in uses of animals in biomedical research has placed great responsibility on support groups charged with providing high quality research animals to investigators. In discussing animal support facilities, staff, and procedures currently in effect at the Yale School of Medicine, it is hoped that better appreciation of this complex problem may be obtained by investigators not directly involved in these activities. Other speakers at this conference will discuss specific needs and demands of investigators for high quality animals; this paper will only deal with selected methods which may help achieve these goals. The professional staff in our group includes 3 veterinary pathologists; 1 virologist; 1 clinical chemist; 1 veterinary clinician and a physiological psychologist. Additional support laboratories operated by this group include radiology, surgical facilities, hematology, and bacteriology. A total of 14 laboratory technicians are directly involved in these areas. Discussion of selected disease outbreaks in our colonies will serve to illustrate how these laboratories and staff function, i.e., sialodacryoadenitis in rats with seroconversion, Sendai virus outbreaks with high morbidity and mortality in suckling mice, latent herpes-like virus infection in guinea pigs, and importance of a specific diagnosis in management (nocardiosis versus tuberculosis). The role of these laboratories in the expanded context of a service resource to the medical school faculty and as investigative laboratories will also be discussed.

INTRODUCTION

Animal research space at the Yale School of Medicine encompasses over 90,000 sq. ft. with an additional 34,000 sq. ft. of buildings located on two research farms about 10 miles from the campus. The daily census ranges from 30,000 to 40,000 animals. Production of inbred and outbred stock exceeds 300,000 animals annually, with an additional 120,000 animals purchased from outside sources. The basic animal research space is made up of one, two and three corridor systems,[1] barrier-maintained facilities, germ-free isolators, isolators and negative pressure rooms for infectious disease experiments, radio-shielded rooms and cubicles for sensitive recording and radio-telemetry experiments, and essential support services (i.e., animal surgery, radiology, and veterinary diagnostic laboratories).

METHODS

The role of veterinary diagnostic laboratories is largely dependent upon the type and quality of animal research conducted within the institution. If a great deal of emphasis is placed on using disease-free animals maintained in a high state of health, then means must be established to determine their health status. Furthermore, once healthy animals are available they must be maintained free from exposure to unwanted animal pathogens during an experiment.

The clinical veterinarian (laboratory animal medicine specialist) must provide major input into this system. It is his/her responsibility to aid the investigator in obtaining disease-free animals, to suggest the appropriate housing system compatible with the research protocol, to maintain the animals disease free, and to document, when necessary, the health status of the animal colonies for additional input into the investigator's research data. If evidence of disease is present, investigative and corrective measures must be instituted. The mechanism to achieve the above goals at Yale has been to develop veterinary diagnostic laboratories in pathology, microbiology, hematology, clinical chemistry and radiology, staffed by faculty members and experienced laboratory technicians who can provide information to the clinical veterinarians and investigators for appraisal and action.

The diagnostic laboratories also provide a service function for investigators who require

* Section of Laboratory Animal Sciences, Yale University School of Medicine, New Haven, Connecticut.

laboratory support in these areas. Thus, the role of these laboratories takes on an expanded significance by providing services extremely difficult to obtain in most institutions through regular human diagnostic laboratories. This resource is, of course, not limited to the scientists outside of these laboratories, but can be developed as an integral part of a faculty member's research laboratory, when applicable. This is certainly the case at Yale and this combined use and manner of staffing is one solution to the problem of establishing quality service laboratories. Table I lists the routine clinical chemistry procedures ordered either by the clinical veterinarian, the veterinary pathologist, or investigators requiring analysis of animal tissues or fluids.

The relationship of these laboratories to each other, the referring veterinary clinician or scientist, and the research disciplines of the faculty of Section Laboratory Animal Sciences (SLAS) are diagrammed in Figure 1.

The mechanical aspects of processing and reporting data and of information retrieval will not be described in detail (see related papers in this symposium). Briefly, however, requests for pathology usually come through the veterinary staff. Provisional diagnostic reports are usually returned to the investigator and the veterinary clinician within 24 hrs. Further processing of material, i.e., serology, virology, bacteriology, clinical chemistry, histopathology, depends on

TABLE I.—*Procedures that can be Ordered through the Diagnostic Clinical Chemistry Laboratory*

Clinical Chemistry	
Protein Electrophoresis	Glucose
Immuno-Electrophoresis	Blood Urea Nitrogen
Gas Chromatographic Patterns	Creatinine
Acid Phosphatase	Uric Acid
Aldolase	Sodium
Creatinine Phosphokinase	Potassium
Isocitric Dehydrogenase	Chloride
Phosphohexose Isomerase	Bilirubin, Total
Ribonuclease	Bilirubin, Free
Paper Chromatography of Sugars	Bilirubin, Conjugated
Paper Chromatography of Amino Acids	SGO Transaminase
Glycoproteins, Protein Bound Hexose	SGP Transaminase
Seromucoid, Hexoseamines, etc.	Alkaline Phosphatase
Total Protein	Fatty Acids
A/G Ratio	Cholesterol
Inorganic Phosphate	
Amylase	
Lipase	
Lactic Dehydrogenase	
LDH Isoenzymes	

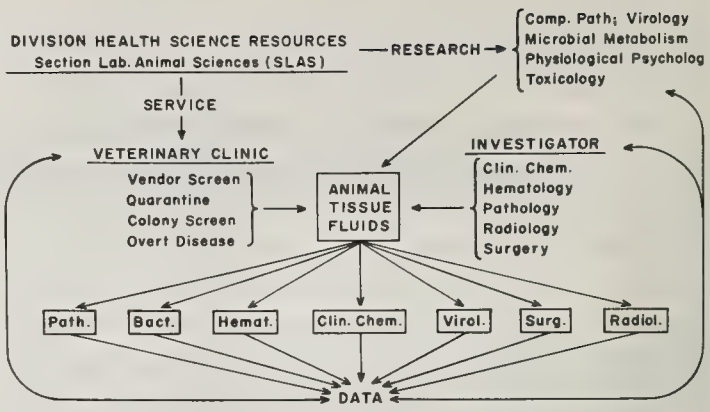


FIGURE 1.—Interactions of Laboratories with Veterinary Clinicians, Investigators, and Research Activities of the Section of Laboratory Animal Sciences.

the judgment of the pathologist and the clinical veterinarian. Final reports are dictated, placed in permanent files, and a final list of diagnoses are sent to appropriate people. This close working relationship is the key to successful application of data generated from the diagnostic laboratories. Straightforward requests for services, i.e., hematology, clinical chemistry, etc., are processed routinely and reports are sent to the requesting individual. A partial review of the diagnostic activities of these laboratories over the past few years lists over 35,000 chemistries and 13,000 necropsies processed to date.

The research interests of most of our faculty in the Section of Laboratory Animal Sciences (SLAS) usually relates directly to the animal colonies. Natural disease of research animals represents an unfortunate occurrence, but the alert investigator may turn this event into a potential source of meaningful research information. Not only can the natural history of diseases be studied, which may have applied benefits to disease control, but important animal model systems may be recognized and characterized. The remaining portion of this paper deals with a few disease problems encountered at Yale and their possible relevance to the subject of this symposium, Research Animals in Medicine.

An explosive, infectious disease causing high mortality in suckling mice between the ages of 1–19 days with peak mortality between 10–14 days was experienced in our closed barrier-maintained ICR mouse production colony.[2] This

colony normally experiences almost no mortality in sucklings, with litter size frequently exceeding 10 animals. Sendai virus was isolated from sick mice during all stages of the outbreak. Serologic studies of the colony indicated low sporadic levels (5–10%) of antibodies (1:10–1:20) to Sendai from 1969 onward; but during and following the outbreak, seroconversion occurred rapidly and resulted in titres up to 1:320 with over 80% of samples positive as seen in Table II. Epizootologic studies (Figure 2) indicated a rapid rise in numbers of litters with some mortality during the first days of the outbreak. A sharp drop then occurred with an end of mortality at 24 days after the first deaths. This pattern correlated inversely with a rise in antibody titres to Sendai virus. Lesions were confined to pulmonary tissues and were compatible with experimental studies of Sendai infection in mice.[3] The sudden occurrence of mortality in an endemic Sendai virus-infected colony is unusual[4] and places new importance on interpretation of low levels of Sendai H.1. antibodies in colonies of mice.

TABLE II.—*H1 Antibodies for Sendai Virus in Sera of Retired Breeders from Nucleus Colony*

Date	H1 Antibody Titers			Total No. of Samples
	<1:10	1:10	>1:10	
1970	60	0	3	63
Pre-Feb. 1971	24	0	2	26
Outbreak Jan. 19, 1971				
Post Feb. 11, 1971	10	2	69	81

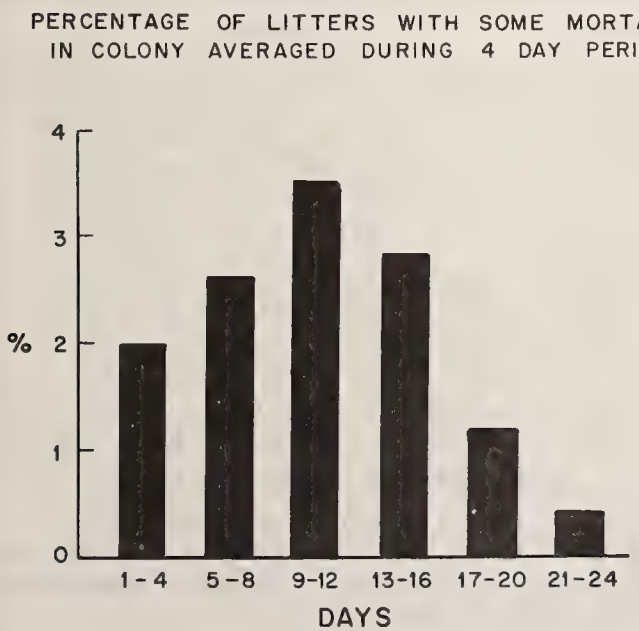


FIGURE 2.—Calculated as litters with one or more deaths out of susceptible litters in colony.

During the course of attempting to isolate an agent associated with lesions of interstitial nephritis from high leukemia strain II guinea pigs, a Herpes virus was isolated in our laboratory. This agent was also isolated in another laboratory by Hsiung and Kaplow.[5] Further work by our group,[6] however, revealed that this agent was widespread not only in strain II, but also in low leukemic strains such as Hartley and random source guinea pigs. Preliminary trials did not demonstrate any causal relationship between this virus and nephritis. The significance of this agent is at present not known, but its possible presence as a latent agent in guinea pigs must be considered in evaluating research data from that species.

Sialodacryoadenitis (SDA) is a highly infectious agent of rats that is associated with a high morbidity but minimal mortality, and which primarily effects the submaxillary salivary and Harderian glands, anterior uveal tract of the eye, and less commonly the parotid and exorbital glands. Jonas et al.[7] studied the pathogenesis of this lesion by light and electron microscopy after infecting germfree rats with crude suspensions of infected salivary tissue. A virus was isolated by Bhatt et al.[8] after inoculating 1-day-old mice intracerebrally and inoculating kidney cell monolayers derived from DA inbred rats. Characterization studies indicate that it is a corona virus, which cross reacts with mouse hepatitis virus (MHV) and is possibly different from Parker's rat corona agent.[9] This disease is widespread in many commercial breeding facilities as shown in Table III. It is of extreme importance that workers using rats for

TABLE III.—*Prevalence Neutralizing Antibody to SDA Virus in Sera * of Rats Obtained from Commercial Sources*

Source	No. Tested	No. Positive
A	12	12
B	12	12
C	12	12
D	12	12
E	12	11
F	12	12

* Parker's Corona virus or as yet other unknown agents may be contributing cross-reacting antibodies.

TABLE IV.—*Neutralizing Antibody to SDA Virus in Sera of WAG-RIG Rats—Retired Breeders*

Time of Collection	No. Tested	No. Positive
Pre epizootic	28	0
Post epizootic	34	33

ocular studies realize that serious damage to that organ may be associated with this disease.[10] Table IV clearly demonstrates seroconversion in a closed WAG/RIG inbred rat colony infected with SDA. This evidence was accompanied by clinical, pathological, and virological evidence of SDA infection.

Simian tuberculosis is a constant threat to research primate colonies the world over. Suspicion that this disease is present in a colony should immediately activate strenuous control measures. Therefore, its improper diagnosis could be most costly. Pulmonary nocardiosis[11] in nonhuman primates was unreported until 1966 when our laboratory described this condition in two unrelated *Macaca mullata*. The similar gross appearance of the pulmonary lesion along with the presence of fragmented, partially acid fast organisms could prompt an incorrect presumptive diagnosis of tuberculosis. Differential features of these diseases are listed in Table V and are now recognized by other laboratories. This example further amplifies the positive role that adequate diagnostic laboratory support can play in defining disease problems and directly effecting disease control.

TABLE V.—*Differential Features of Pulmonary Nocardiosis versus Tuberculosis in the Non-Human Primate*

	Nocardiosis	Tuberculosis
Radiology	Similar except mediastinal widening should not be expected.	Dense infiltrates with frequent widening of mediastinum due to lymph node involvement.
Bacteriology	Gram +, beaded filamentous rods, partially acid fast. May fragment. Aerobic, grows well on Lowenstein agar.	Acid fast, thin, slightly curved rods; best site for in vivo smear and culture is *LARYNX*.
Gross Pathology	Necrotizing bronchopneumonia identical to T.B. No Gohn's complex. No extrathoracic lesions seen to date.	Necrotizing, granulomatous bronchopneumonia. *Invariably* Gohn's complex present. Extrathoracic lesions common.
Histopath	Pleomorphic infiltrates, giant cells not observed. Brown-Brenn stains may reveal individual beaded filaments or colonies of organisms. Partially acid fast. Fibrosis may be present.	Usually mononuclear infiltrates predominate, accompanied by many giant cells. Acid-fast techniques reveal typical rods within cytoplasm of giant cells. Fibrosis usually not seen.
Epizootiology	Sporadic, not considered highly infectious.	Highly infectious, may spread rapidly through colony.

Finally, I would like to mention the facilities of these diagnostic laboratories as resources in recognizing and characterizing potential animal model systems. The cat is frequently found to have medial hypertrophy and hyperplasia of pulmonary arteries (PA) (1.3%–68%).[12] Our experience places this figure at approximately 20–30%. Contrary to recently published work by Rogers et al.,[13] we have found significant elevations of pulmonary vascular resistance (PVR) in 2 cats with natural disease. Pathogenetic studies of this disease indicated that oral inoculation of embryonated *Toxocara cati* eggs or infective larvae of *Aelurostrongylus abstrusus* resulted in production of pulmonary vascular lesions and moderate elevation of PVR.[14] Preliminary findings suggest this inducible vascular disease resulting in elevated PVR could be an exciting animal model system to: study (1) pathogenic mechanisms of vascular smooth muscle changes, and (2) changes in pulmonary hemodynamics not secondary to vascular occlusion or destruction of the vascular bed following parenchymal disease (Figures 3 and 4).

Other systems studied in our laboratory include a fascinating and controversial intestinal

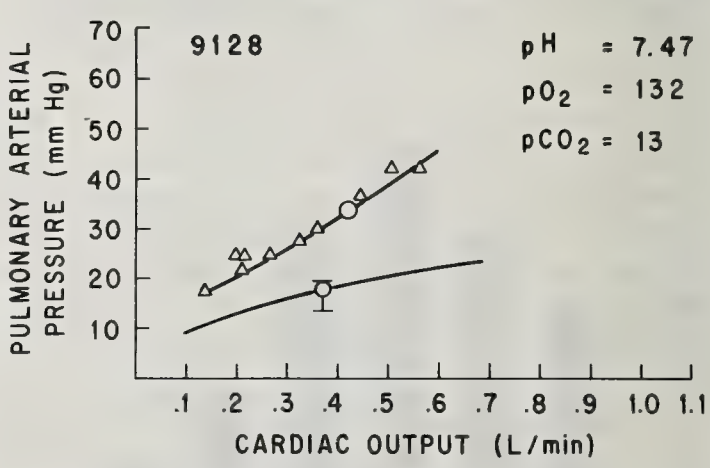


FIGURE 3.—Pressure flow curve from uninoculated cat with lesions of PA medial hyperplasia. Lower curve is from a normal control cat.
O = Flow rate of 125 ml/kg/min.
I = Range within ± 1 S.D. for normal control data calculated at a flow rate of 125 ml/kg/min.

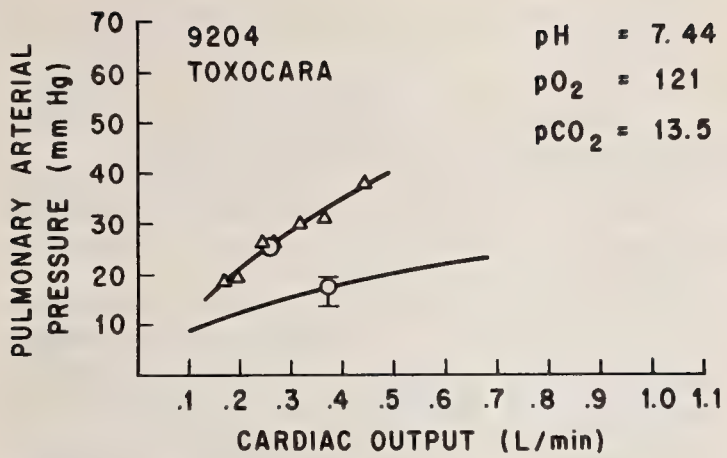


FIGURE 4.—Cat inoculated with 1,000 embryonated eggs of *Toxocara cati* for 20 consecutive days and then studied 4 days later. This pulmonary artery pressure/flow curve demonstrates marked elevation as compared with control data. Lower curve is from a control animal.

O = Flow rate of 125 ml/kg/min.

I = Range within ± 1 S.D. for normal control data calculated at a flow rate of 125 ml/kg/min.

disease of hamsters referred to variously as "wet tail," proliferative ileitis[15] and enzootic intestinal adenocarcinoma.[16] Our original publication[16] suggested the terminal event in this intestinal disease should be considered a neoplastic invasion. Recently, two cases of this disease have been studied[17] with apparent neoplastic lesions in their livers. These hepatic lesions were necrotic except for viable acinar structures at their borders. The question whether these acini are derived from bile ducts or are indeed metastatic from the intestine has not been answered. Heslop[18] reported on a disease outbreak of intestinal colonic adenocarcinoma in the rat, which, in our opinion, has marked similarities to the hamster disease. Both of these examples may be unique models for future study of intestinal cancer or at least remarkable proliferative disorders. The reactive features associated with invading "tumor" tissue in the Hamster's intestinal wall suggests a possible role for immunologic reactivity against tumor antigens in the natural history of the disease.

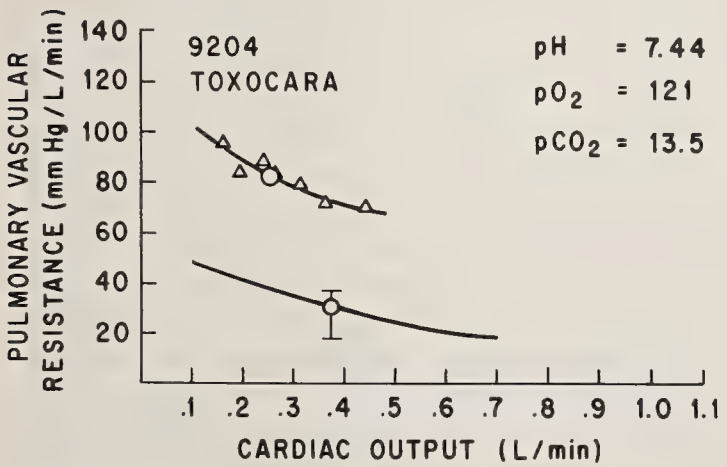


FIGURE 5.—This pulmonary vascular resistance/flow curve of same cat in Figure 4 demonstrates a marked increase in PVR. Vascular lesions were minimal in this case, possibly indicating an early vasomotor component.

O = Flow rate of 125 ml/kg/min.

I = Range within ± 1 S.D. for normal control data calculated at a flow rate of 125 ml/kg/min.

DISCUSSION/SUMMARY

Many other diseases have been studied in our laboratory,[19-21] but the purpose of this paper is to emphasize the many-faceted approaches and rewards that the veterinary diagnostic and investigative laboratory can achieve in its service and research roles within an institutional setting involved with "Animals in Medical Research."

REFERENCES

1. JONAS, A. Laboratory animal facilities. J. Am. Vet. Med. Assoc., 146:600–606, 1965.
2. JONAS, A. M., and BHATT, P. N. A spontaneous outbreak of sendai virus causing high morbidity and mortality in suckling mice. Manuscript in preparation.
3. APPELL, L. H., KOVATCH, R. M., REDDECLIFF, J. N., and GERONE, P. J. Pathogenesis of sendai virus infection in mice. Am. J. Vet. Res. 32:1835–1841, 1971.
4. PARKER, J. C., TENNANT, R. W., WARD, T. G., and ROWE, W. T. Enzootic sendai virus infections in mouse breeder colonies within the United States. Sci. 13:936–938, 1964.
5. HSIUNG, G. D., and KAPLOW, L. S. Herpeslike virus isolated from spontaneously degenerated tissue culture derived from leukemia susceptible guinea pigs. J. Virol. 3:355–357, 1969.
6. BHATT, P. N., PERCY, D. H., CRAFT, J. L. and JONAS, A. M. Isolation and characterization of a Herpeslike (Hsiung-Kaplow) virus from guinea pigs. J. Inf. Diseases, 132:178–189, 1971.

7. Jonas, A. M., Craft, B. S., Black, C. L., Bhatt, P. N., and Hilding, D. A. Sialodacryoadenitis in the rat. A light and electron microscopic study. Arch. Path. 88:613–622, 1969.
8. Bhatt, P. N., Percy, D. H., and Jonas, A. M. Characterization of the virus of Sialodacryoadenitis of rats. A new member of the corona virus group. J. Inf. Diseases, 126: 123–130, 1972.
9. Parker, J. S., Cross, S. S., and Rowe, W. P. Rat corona virus (RCV); A prevalent, naturally occurring pneumotropic virus of rats. Arch. Ges. Virus 31:293–302, 1970.
10. Jonas, A. M. Unpublished data.
11. Jonas, A. M., and Wyand, D. S. Pulmonary nocardiosis in the Rhesus. Importance of differentiation from tuberculosis. Pathologica Veterinaria 3:588-600, 1966.
12. Tashjian, R. J., Das, K. M., Palich, W. E., Hamlin, R. L., and Yarns, D. A. Studies on cardiovascular disease in the cat. Ann. N. Y. Acad. Sci. 127:581–605, 1965.
13. Rogers, W. A., Bishop, S. P., and Rohorsky, M. W. Pulmonary artery medial hypertrophy and hyperplasia in conventional and specific-pathogen-free cats. Am. J. Vet. Sci. 32:767–774, 1971.
14. Jonas, A. M., Swerczek, T. W., and Downing, S. E. Vaso-occlusive pulmonary artery disease in the cat. A preliminary report. Acta. Radiologica (in press).
15. Boothe, A. D., and Cheville, N. F. The pathology of proliferative ileitis of the golden syrian hamster. Path. Vet. 4:31–44, 1967.
16. Jonas, A. M., Tomita, Y., and Wyand, D. S. Enzootic intestinal adenocarcinoma in hamsters. Spec. Wildlife & Lab. An. issue of J. Am. Vet. Med. Assoc., Nov. 15, 1965.
17. Jonas, A. M., Jacoby, R. O., Wyand, S., and Cornog, J. L. The natural history and pathogenesis in hamsters of an apparent infectious disease resulting in intestinal adenocarcinoma in a select group. Manuscript in preparation.
18. Heslop, B. F. Cystic adenocarcinoma of the ascending colon in rats occurring as a self limiting outbreak. Lab. Animals 3:185–195, 1969.
19. Jonas, A. M., and Carrington, C. Vascular patterns in primary and secondary canine pulmonary neoplasm. A study employing cast corrosion and gelatin injection preparations. Am. J. Path. 56:79–95, 1969.
20. Jonas, A. M., Percy, D. H., and Craft, J. L. Tyzzer's disease in the rat—its possible relationship with megaloileitis. Archives of Path. 90:516–528, 1970.
21. Jonas, A. M., and Hukill, P. Histogenesis of pulmonary adenocarcinoma in the cat. Arch. Path. 85:573–579, 1968.

THE RESEARCH DOG: RANDOM SOURCE OR COLONY REARED?

S. Potkay and J. D. Bacher*

Although unparalleled advances have been made in research laboratory instrumentation and methodology during this century the standard research dog of today is essentially the same as its counterpart of 50 or 100 years ago. As an alternative to the continued use of dogs of unknown genetic background, age, temperament and disease experience, The National Institutes of Health and various other facilities have undertaken to breed dogs selected to meet specific investigational requirements. Although such animals are generally more expensive on an individual basis than those obtained from vendors, it has been shown that overall needs for dogs can be reduced by their use. This presentation will summarize the experiences of the NIH Animal Center in breeding and rearing American foxhounds for research as compared with quarantining and conditioning random source dogs. The use of a canine blood donor colony as a means of reducing the total needs for dogs will also be discussed.

INTRODUCTION

On a national basis, more than a quarter of a million dogs are used in research annually (Table I).[1-3] Of these, at least 85% originate from either pounds or animal dealers or both. Relatively few (1.3%) are donated to medical institutions for research. These data also indicate a growing tendency for dealers and research institutions to breed dogs specifically for research. It is the purpose of this communication to discuss previous and current practices at The National Institutes of Health as they relate to procuring, quarantining, and conditioning of random source dogs and to suggest alternatives to this method of supplying the needs of the research community for dogs.

RANDOM SOURCE DOG COLONY

Random source dogs, for the most part, consist of unowned "wild" dogs, rejected hunting dogs and strayed or unwanted pets.[4] They may be mongrel or purebred, and are in various states of health. The various steps required to bring the random source dog from its original setting into the laboratory are shown in Table II. The minimum period of time involved in these multiple transfers is approximately 10 days, but the average is probably about 30 days. Exposure of the dogs to a variety of pathogens at each facility involved and stress stemming from frequent transportation and changes in social grouping, diet, husbandry and environment are considered principal factors responsible for the morbidity and mortality observed among random source dogs at the research facility. These observations are illustrated by the data shown in Figure 1. During fiscal year 1965 through 1967, dogs accepted for quarantine and conditioning suffered a combined mortality/morbidity of about 65%.[5] It was recognized that 30 days were frequently insufficient to adequately condition dogs for investigational use. Consequently, during fiscal year 1968 through 1970, the quarantine and conditioning period was extended to 45 days; coincidentally, criteria for accepting dogs into the facility were reformulated and adhered to more strictly. These actions were associated with a slight decrease in morbidity and a substantial increase

TABLE I
APPROXIMATE NUMBERS OF DOGS USED FOR BIOMEDICAL RESEARCH IN THE UNITED STATES*: 1967-1969

Year	Sold†	Bred‡	From Pounds†	Donated	Total
1967	247,753	17,599	105,672	–	371,024
1968	269,584	32,417	107,471	–	409,472
1969	220,546	43,723	103,028	4,932	372,229
Totals	737,883	93,739	316,170	4,932	1,152,725

* Adapted from ILAR News[1,2,3]

† Based on an average 63.2% return of questionnaires

‡ Based on an average 59.9% return of questionnaires

* Veterinary Resources Branch, Division of Research Services National Institutes of Health, Bethesda, Maryland.

TABLE II

FACILITIES AND TIME INVOLVED IN "PROCESSING" RANDOM SOURCE DOGS

Type of Facility	Approximate Time in Facility
Pounds, Auctions and "Bunchers"	5 to 14 days
Class B, USDA Licensed Animal Dealer's Facility	5 to 30 days or more
Research Facility: Quarantine and Conditioning	0 to 21 days or more
Research Laboratory	0 to indefinite period

in mortality. It should be noted, however, that many of the deaths occurring during this period were related to an epizootic of *Streptococcus zooepidemicus*. The overall result of the 45-day quarantine and conditioning period, however, was the issuance of dogs which were in optimal condition.

From fiscal year 1971 to the present, we have been purchasing 30-day preconditioned random source dogs and quarantining them for an additional 15 days. Reduction of the conditioning period at the NIH was undertaken to reduce space requirements for random source dogs and thus permit increased production of colony bred dogs. The benefits accruing to the conditioning facility and the NIH researcher by this arrangement are shown in the third pie-graph (Figure 1). Only 9.0% of the dogs accepted became ill; the remaining 91.0% remained clinically healthy. It must be understood, however, that this kind of arrangement merely shifts the period of major morbidity/mortality to the dealer's facility: it does not alter the exposure and disease experience of dogs destined for biomedical research. The specific quarantining and conditioning procedures and various factors identified with mortality and morbidity among random source dogs in this facility are discussed elsewhere.[6]

CANINE BLOOD DONOR COLONY

The majority of the dogs issued for research at the NIH are used by The National Heart and Lung Institute (the annual average is 88% of the total number issued; the range is from 85% to 92%). Prior to 1967, many of these dogs were exsanguinated to obtain canine blood required to prime heart-lung machines for experimental surgery and for a variety of specific organ perfusion experiments. In 1967, a pilot study was initiated to evaluate the feasibility of meeting research needs for canine blood using a permanent group of dogs as donors. Although resistance was encountered initially, familiarity with the product and its ready accessibility has resulted in a sustained demand by researchers for canine blood from this source. The subsequent establishment of a canine blood donor colony of adequate size to meet all requests for blood has helped to reduce the average annual use of random source dogs.

Certain basic routines have been developed for canine blood donors. Blood collection techniques are evaluated periodically to insure that sterility of the product is maintained. All dogs in the colony are given physical examinations at periodic intervals, and prior to blood collection, each dog is evaluated hematologically and clinically. They are examined twice a year for helminth parasites and treated as necessary; immunizations against the common canine diseases are administered annually. Individual records are maintained on the dogs pertaining to their clinical and blood donation histories. The blood group of donors must be A-negative and they must be negative for microfilariae. Donors must be of an adequate size (28 kg or more) to provide 500 ml (1 unit) of normal blood every

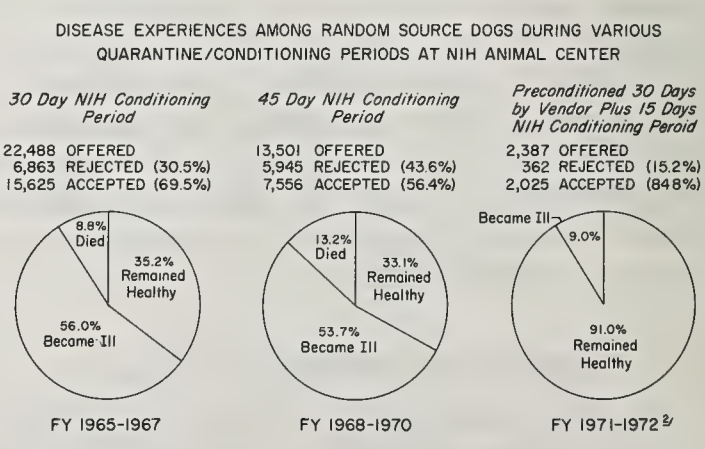


FIGURE 1.

three weeks.[7] On such a schedule, each donor can supply 17 units of blood per year. Based on an average yield of two units of blood per exsanguinated random source dog, each permanent donor thus represents a savings of 8.5 dogs that would otherwise have to be exsanguinated. The current colony of 240 donors, therefore, can produce a quantity of blood equal to 2040 exsanguinated dogs per year.

CANINE BREEDING COLONY

Dissatisfaction with the quality of random source dogs led to establishing a production colony of purebred foxhounds to provide standard research dogs for the NIH researcher. Some of the advantages of the colony bred research dog include: known age and genetic background and acceptable temperament. In addition, morbidity and mortality associated with common canine diseases, including helminth and protozoan parasitisms, can largely be eliminated. Production of uniform, purebred dogs also lends itself to ease in assembling baseline reference data pertaining to anatomy, physiology hematology and biochemistry for specific sex, age or other groups. Finally, litter-mates or other closely related dogs can be made available to meet specific investigational needs.

The argument generally employed against the use of dogs specifically bred for research is that their initial purchase cost is more than that for random source dogs. While this is true, it is an oversimplification of the situation, for it fails to take into consideration all of the expenses associated with utilizing dogs in research.

Itemized operative and postoperative expenses for open heart experiments at one research facility indicated that the cost of the dog was only 6.7% of the total expense.[8] Considering that overhead, professional salaries and related expenditures (e.g., clinical pathology, necropsy, photography, etc.) were not included in arriving at this figure, the cost of the dog becomes an even less significant factor. Another important consideration is the stamina and viability of the research dog. Reported observations that the survival rate of Laborador retrievers was substantially higher than that of healthy, conditioned random source dogs undergoing open heart surgery[8] are similar to opinions expressed by NIH researchers using purebred foxhounds and random source dogs. In this regard, arguments against using purebred dogs become even less tenable.

Acceptance of the American foxhound as a standard research dog at the NIH seems well established and current demands exceed production. The original pilot canine breeding colony has been gradually expanded and presently consists of 130 bitches and 7 stud dogs. Production has increased to the extent that the number of colony bred foxhounds issued exceeds that of random source dogs.

CONCLUSIONS/SUMMARY

The interaction of the three programs described above is illustrated in Figure 2. The graph, which shows the numbers of dogs issued and those maintained for production purposes, demonstrates the sharp decline in the use of random source dogs and in the overall numbers of dogs required to meet the total needs of the NIH. In evaluating these figures, it should also

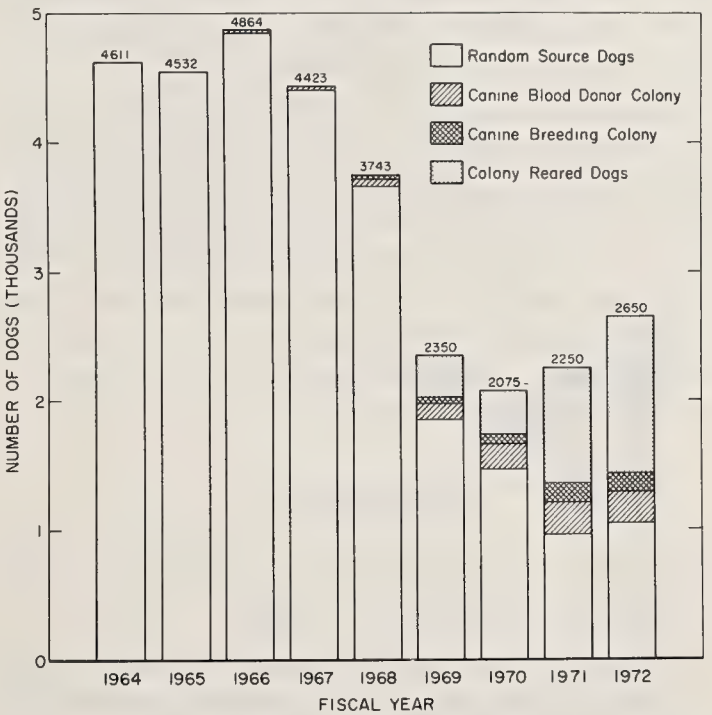


FIGURE 2.—Total numbers of dogs maintained and issued for biomedical research at the National Institutes of Health; fiscal years 1964–1972.

TABLE III

A COMPARISON OF RANDOM SOURCE AND COLONY-BRED DOGS AND CANINE BLOOD ISSUES AT THE NATIONAL INSTITUTES OF HEALTH FROM FY 1964 THROUGH 1972

Fiscal Year	Random Source:		Canine Blood:		Colony Bred:		Total
	Dogs	%	Dogs*	%	Dogs	%	
1964	4611	100.0					4611
1965	4532	100.0					4532
1966	4839	100.0					4839
1967	4398	96.9	133	3.1			4531
1968	3618	94.2	222	5.8			3840
1969	1861	61.2	860	28.3	320	10.3	3040
1970	1466	42.9	1620	47.8	328	9.3	3414
1971	965	30.1	1367	42.7	872	27.2	3204
1972†	1060	29.6	1312	36.7	1210	33.7	3582
Totals	27350	77.0	5514	15.4	1210	7.6	35653

* 1 dog = 2 units of blood
† Dog and blood issues from July through December, 1971 multiplied by 2

be recalled that the breeding and donor colonies are relatively permanent entities.

The impact of blood issues from the donor colony on the random source and breeding programs is shown in Table III. Blood issues are expressed in terms of dog-equivalents for ease in relating them to overall dog use. In addition to reducing random source dog issues, the blood donor program originally served to make facilities available for expanding the breeding colony to its present size. The firm establishment and continued enlargement of the breeding colony is expected to further reduce the need to quarantine and condition random source dogs.

Research institutions concerned with reducing the numbers of dogs required to meet their program needs and with increasing the quality of the dogs used might consider adapting either or both of the alternatives described above. The ultimate suitability of instituting either blood donor or canine breeding activities will, of course, depend on the specific requirements of the individual institution.

REFERENCES

1. *Institute of Laboratory Animal Resources News*, National Research Council—National Academy of Sciences. Vol. XII, No. 1: ii, 1968.
2. *Institute of Laboratory Animal Resources News*, National Research Council—National Academy of Sciences. Vol. XIII No. 1: iv, 1969.
3. *Institute of Laboratory Animal Resources News*, National Research Council—National Academy of Sciences. Vol. XIV No. 2: vi, 1970.
4. JONES, E. M. The laboratory animal welfare act: regulations, standards, enforcement, and progress. Journ. Amer. Vet. Med. Assoc. 153:1874–1877, 1968.
5. ZINN, R. D. The research dog. Journ. Amer. Vet. Med. Assoc. 153:1883–1886, 1968.
6. PALMER, A. E. Disease problems encountered during conditioning of random source dogs and cats. Proceedings of the National Conference on Research Animals in Medicine, 1972.
7. POTKAY, S., and ZINN, R. D. Effects of collection interval, body weight and season on the hemograms of canine blood donors. Lab. Animal Care 19:192–198, 1969.
8. FLETCHER, W. D., HERR, R. H., and ROGERS, A. L. Survival of purebred Laborador retrievers versus pound dogs undergoing experimental heart valve replacement. Lab. Animal Care 19:506–509, 1969.

DISCUSSION

C. STORMONT, School of Veterinary Medicine, Davis, California: Dr. Potkay, when you're figuring the cost of your dogs and your select breeding colony, do you figure in your own salary, too?

DR. POTKAY: Yes.

DR. STORMONT: And all the caretakers?

DR. POTKAY: Yes.

DR. STORMONT: What was the cost over and above, say, random bred dogs? About $8 more?

DR. POTKAY: Oh no.

DR. STORMONT: Six?

DR. POTKAY: This slide will give you an idea of what we do charge for these various dogs.

DR. STORMONT: $22.

DR. POTKAY: Right.

DR. STORMONT: Well, what's the average price of your dogs over nine months old? Are they random source dogs?

DR. POTKAY: These are random source dogs that we issue and regardless of size or age, they're $99.50.

DR. STORMONT: Most of those you're selling are all over nine months of age, aren't they?

DR. POTKAY: No, a lot of them of various ages are used in this category.

DR. STORMONT: I have one more question. Why do you exsanguinate random bred dogs when you bleed them, and don't exsanguinate your own dogs when you bleed them?

DR. POTKAY: We were never in the business of supplying blood at all until we initiated

our donor colony. Prior to that time, the investigators asked us for dogs. They requested either operating dogs, table dogs, surgical dogs, or donor dogs. The dogs were sent to their laboratories and were exsanguinated there. Much of the resistance that we had initially even to supplying blood was related to the fact that investigators would not accept blood that was drawn somewhere else and had to be shipped and maybe spend about three or four hours in transit. They said this blood would be no good. I think that just the converse has proven true here.

DR. STORMONT: Very good. Are you breeding your colony dogs now? To simplify the matter, why don't you eliminate the A red cell factor? Just get it out of your colony?

DR. POTKAY: We have done that in our breeding colony.

QUESTIONER (unidentified): We have trouble with the personality of the colony-reared dogs in comparison with the random source dogs. I wondered if you have any comments about making these dogs more practical for us?

DR. POTKAY: Well, that's a problem that we have encountered also. The only thing that seems to work in our situation is to have the animal caretakers handle the dogs as frequently as possible. The puppies have to be handled and played with on occasion. They have to be taken out of their individual kennels, put on leashes, and walked around a bit. If you don't do that and keep them in the same cage or kennel in which they were born, then they become terribly withdrawn. Some of them become a little more aggressive than would be desirable. We find that only by continuous handling can we make them of acceptable temperament.

J. MOOR-JANKOWSKI, New York University, New York: I was quite interested in your answer to the question of Dr. Stormont, which I did not completely understand. Are all factors included in the price of the dogs? Is this a completely self-supporting enterprise or do you have subsidies?

DR. POTKAY: As far as we know, this represents a fair price. We get our figures from the financial management people. Obviously, we have a number of accounts. The numbers and figures are maintained at a central area, and periodically we're advised as to whether the dogs are costing more or less than what we're actually charging for them.

E. I. GOLDSMITH, Cornell Medical Center, New York: I think that the matter of the cost of the random source dog is a terribly important point. In New York, which is generally regarded to be the perhaps the highest cost of living area in the country, we are being charged approximately 30 per cent less for a random source dog than you are charging, which seems to me to clearly emphasize the fact that many animal care facilities throughout the country are receiving hidden subsidies of which all of us should be aware. The institutions we are associated with have a heavy investment in the animal care facilities that is not reflected in our individual project grants and the prices we're paying are very unrealistic in terms of the animals we're purchasing.

A SUPPORT LABORATORY FOR A MEDICAL SCHOOL ANIMAL RESEARCH FACILITY

Ward R. Richter*

Concurrent with the development of medical research and the use of large numbers of laboratory animals in research, a need for improved animal resources has arisen. Major universities have established support laboratories to improve animal health and animal care. These laboratories with their staff of animal health specialists are uniquely suited to contribute to the three major functions of the medical school: service, research and teaching. The support laboratory for the A. J. Carlson Animal Research Facility at the University of Chicago is an example of one which operates within this context. The facilities, operation and contributions of this laboratory are described.

INTRODUCTION

In recent years there has been a trend toward centralization of animal facilities at medical schools or the centralization of administrative functions related to animal care at these institutions. A central facility can provide specialized support for animal research including the provision of health services. An outgrowth of this trend has been the establishment of laboratories for the support of health care in many central animal care programs. They were established because a diagnostic and support need was recognized; however, the diagnostic service is only a small part of the contribution which they can make to the mission of a university. Ideally, a laboratory can contribute to the three major activities of a medical school: teaching, research and service.

The basic mission of a university or medical school is one of education. Medical service and biomedical research are important goals of society on their own and can exist independently and outside of the context of a university community but medical schools engage in these two activities because they are essential to the educational process. Medical service provides the experience necessary for the training of future physicians and biomedical research provides the training for future scientists and teachers as well as the environment and stimulation for academic excellence on the part of the teaching staff.

A support laboratory for an animal facility should and must function and make its contribution within this context. It can do this indirectly without participating in the teaching process by providing superior service and thus support to the research activity of a school, ultimately strengthening its education role. Alternatively, it can engage in teaching and or research activities directly, the role chosen for a laboratory in any particular institution depending on the local requirements and capabilities of the available staff. However, a laboratory can contribute significantly and efficiently to each of the three basic activities in such a way that teaching, research and service are each supported by the others and performed better than either of them alone.

The staff of a good animal diagnostic and support laboratory compliments or adds to the teaching program of a medical school at several levels. A very practical and essential educational role involves training in animal handling and research techniques for animal caretakers, animal technicians, investigators and student investigators. Historically, graduate training has included courses or special training in the use of instruments and techniques such as electron microscopy or histochemistry, but similar formal training in the selection and use of laboratory animals has often been inadequate. Young investigators may select an animal species for no other reason than tradition with little or no awareness of its limitations or applications to his research problem. It has not been unusual for young investigators to be temporarily frustrated in their work because of the appearance of unexpected spontaneous dis-

* University of Chicago, Chicago, Illinois.

ease or unexpected species variations. Thus, lectures, seminars and courses in comparative medicine and pathology are desirable at the undergraduate and the graduate level. In addition, an animal support laboratory is ideally suited to sponsor graduate study or residency training in comparative pathology, laboratory animal medicine or the other specialized areas of comparative medicine.

Research conducted in these laboratories naturally follows the areas in which the staff has interest and skill but there are some specific areas and approaches that are particularly appropriate to their background and expertise. One area for research is the study of laboratory animal disease directed toward elimination or control of spontaneous disease to provide a more uniform laboratory animal and less variation in experimental results. The most significant area for potential contributions to medicine is the study of animal models for human disease. Careful diagnostic work with recording and retrieval of data often leads to the discovery of new models, but an even more productive approach is the experimental manipulation of animals to develop effective models. To be useful, a potential model must be defined in detail with physiologic and pathologic parameters evaluated, a form of research for which an animal laboratory is well-suited. As research becomes more sophisticated, the normal and limits of normal variation need to be defined precisely. Spontaneous disease and the effects of aging must be recognized and evaluated. In other words, a statistically normal animal must be defined in contrast to a teliological normal experimental animal.

The service function is involved in the diagnosis of animal disease, in quality control of animals and in defining the baselines of spontaneous disease in a colony. Staff members are valuable consultants to other investigators or they can serve as important members of research teams.

These three functions are so interrelated that it can be difficult to separate them. The diagnostic case material serves as training material for the students and as a source of potential animal models. Research contributes to improved diagnosis, elimination of disease and training of students. The teaching increases the expertise of the staff and the students can have strong input into the diagnostic service.

UNIVERSITY OF CHICAGO SUPPORT LABORATORY

The animal support laboratories at the University of Chicago are one example of a laboratory which relates to the medical school in the way just described and by way of example illustrates the role such a laboratory can play in support of a medical school. The laboratory facilities are housed in the A. J. Carlson Animal Research Facility which occupies 75,000 square feet of floor space. The Carlson Animal Facility is a little over three years old and the support laboratory with its staff has only been in existence since that time. The laboratory has outlined a four-fold mission: (1) to serve as a diagnostic center for diseases of laboratory animals in the A. J. Carlson Animal Research Facility and the numerous other animal facilities of the University of Chicago; (2) to serve similarly as a diagnostic consultant and referral center for biological and medical investigators in the Chicago area; (3) to conduct research to enhance the art and science of laboratory animal and comparative medicine; and (4) to provide graduate or specialty training in comparative pathology or medicine.

The basic laboratory is partially supported by PHS Animal Resource Grant no. FR–00409. Additional support comes from several other PHS research grants for specific research projects and from university funds. The students are supported by various divisional training funds and individuals fellowships.

Facilities and Equipment

The laboratories consist of two necropsy rooms, a histology laboratory, a clinical laboratory for hematology, urinalysis, parasitology and clinical chemistry, a microbiology laboratory, three laboratories for comparative biochemistry and an electron microscopy suite. Additional supporting facilities include a surgical suite and a radiographic facility. Various physical examination, treatment and quarantine

rooms are available for large and small animal medical problems.

At the present time the staff includes eight individuals with a D.V.M. degree, one with an M.D. degree and two with Ph.D. degrees. They contribute all or a part of their effort to one or more of the four basic missions of the program. In addition, there are four veterinarians who are graduate students and one M.D.–Ph.D. student contributing heavily to the operation of the support laboratory. Eight to ten full-time technicians and several part-time technicians are employed. The professional staff has specialty training or graduate training in laboratory animal medicine, veterinary and comparative pathology, oncology, radiology, radiobiology, pharmacology and toxicology. Many technical skills are represented in the group.

Service Activities

Two of the missions of the laboratory are service in nature. This includes the diagnostic support of the animal facilities of the University of Chicago and the diagnostic referral center for other investigators in the Chicago area. The services provided are listed in part as follows:

1. Post-mortem evaluation is done on all animals which die in the facility and the depth of the examination is determined by the pathologist in consultation with the clinical veterinarian or the investigator. Clinically ill animals are sacrificed for post-mortem evaluation when indicated.

2. Selected specimens of new shipments of animals, especially rodents, are examined to determine the base line of spontaneous disease and as an aid in evaluating their health status during the quarantine period.

3. Post-mortem examination is performed on selected control animals at the end of experimentation (with cooperation of the investigator) to determine the disease status of these animals after housing in the facility for a period of time. This is compared to their status at time of arrival to evaluate the effectiveness of disease control in the facility.

4. The microbiology service conducts a limited survey of infections and their antibiotic sensitivity in the various species of animals. This service is not fully developed but we have just initiated a program to survey the bacterial contaminants of the rooms, cages, water bottles and utensils used in the different sections of the animal facilities.

5. The clinical laboratory is used for clinical diagnosis and as a quality control for the conditioning of dogs, cats and primates. Before release from quarantine all of these species are given a complete clinical work-up and at various times during the quarantine, selected clinical tests are utilized as an aid in the control of parasites and infectious disease.

6. A valuable service of the staff is in relation to consultation with investigators on the use of animal models, experimental design, disease control and evaluation of experimental results when complicated by disease. This may include assistance in post-mortem examination and aid in teaching animal techniques.

Research Activities

A partial listing of previous or current research demonstrates what a well equipped laboratory can contribute in an animal facility.

1. The largest and the most productive research effort of the group is a program on the development and definition of animal models for the study of carcinogens and the process of carcinogenesis.

2. Another group has been investigating models for the study of bladder cancer. This has included a comparative study of bladder ultrastructure in many species, and the pathogenesis of tumors produced by dibutylnitrosamine in the urinary bladder. There are significant morphologic differences between primate bladder and rodent bladder suggesting that the potential for a different response to carcinogens exists.

3. The gerbil has been investigated as a possible model for renal carcinogenesis and as a model for the study of the selective toxicity of lead in the kidney. The urine concentrating ability of this species provides some advantages in the study of the mechanisms of lead injury.

4. Basic reactions of the pancreas at the ultrastructural level are being studied to aid in the development of a model for cystic fibrosis, a disease without a known animal model.

5. A major research effort is conducted in cooperation with a member of the Department of Psychiatry on the study of drug addiction and particularly addiction to methamphetamine in animal models. A model is now in use which promises to be valuable in the determination of the mechanisms of development of tolerance to methamphetamine.

6. The diagnostic case material is also valuable as a source of research leads or projects. An outbreak of Tyzzer's disease lead to a study of the ultrastructure of the organism and its relationship to infected hepatocytes in the mouse. The ubiquitous problem of canine distemper in the dog conditioning facility provided the opportunity to elucidate the ultrastructural nature of distemper inclusions in the urinary bladder and their relationship to helices of ribonucleoprotein. The availability of a well-equipped laboratory permits us to take advantage of unexpected spontaneous disease as a source for research projects.

Teaching Activities

The staff is very active in teaching courses in the Departments of Pathology and Radiology and to some extent in Pharmacology. At least four graduate courses are taught or organized by the staff. Staff members also participate by lecturing in other graduate courses and in medical school undergraduate courses. The staff teaches a course, Principles of Comparative Pathology, which is designed to provide insights into the basic mechanisms of disease by a study of the comparative reaction of various animal species to injury. It covers the problems of model selection, transposition of data from species to species, the role of spontaneous disease and the problems of base line data. The body of the course involves a study of the basic principles of pathology on a comparative basis including circulatory, oncologic inflammatory and degenerative disease. A second course is offered by the Department of Pharmacology on the comparative gross and microscopic changes in tissues and organs caused by drugs and toxic agents.

A program of graduate training in comparative pathology is carried on as a part of the experimental pathology training program in the Department of Pathology. The training provides some basic experience in diagnostic pathology and the students are required to rotate through the necropsy service, the microbiology and clinical laboratory as well as to receive diagnostic experience at the Lincoln Park Zoo. This is designed to provide them with basic diagnostic skill in veterinary and comparative pathology, but is not specifically designed to meet American College of Veterinary Pathologists requirements without additional experience.

The basic emphasis of the program is on experimental pathology with emphasis on comparative pathology and the use or development of animal models. Students must meet all of the requirements of the Department of Pathology and they are required to take a number of courses related to human disease to make the training truly comparative. Because of the research interests of the staff, the research projects of the students generally involve a study of the cellular and subcellular response to chemicals including carcinogens. As the support laboratory matures we plan to offer training in a wider area of comparative medicine including radiobiology.

SUMMARY

The program at the University of Chicago is still evolving and has strengths as well as weaknesses but we feel that it has made a real contribution to the University and to Medical Science in general. The support laboratory exists as a service laboratory and for this function its director is responsible to the Director of the Carlson Animal Facility in the same way that a hospital laboratory director is responsible to the director of a hospital. The teaching and research functions are administered by the various academic departments. When a central animal facility was developed, it was decided that there would be no department of comparative medicine or similar administrative structure but rather the staff would receive academic appointments in the existing departments. They then would provide service to the animal facility in the same way that the faculty of the various

departments staff the hospital in addition to performing their academic duties.

The three senior staff members are involved in the direction of the Carlson Animal Facility and in the direction of the laboratories. They all have tenured academic appointments in the Departments of Radiology-Pharmacology, Radiology and Pathology respectively. Other senior staff members of the Carlson Facility have appointments as research associates in several of the departments. An effort was made to have different departments represented, giving the several departments a selfish interest in the success of the animal facility and its programs. More important, it develops a commitment to the departments by the facility staff and it leads to a better appreciation for the other investigators' problems and to real communication with the whole medical school. It has insured the academic excellence of the staff because appointments must meet the criteria of the academic departments on their own terms. Interdepartmental cooperation on projects is encouraged and common at the university so there is a strong tradition for members of various departments to work together as this group does in the animal research facility. Thus, we have had no administrative or traditional barriers to break down with the various departments staffing the facility.

DISCUSSION

E. I. GOLDSMITH, Cornell University, New York: Rather than a question, Dr. McPherson, I think it might be appropriate to make a comment at this time because I don't think the previous speakers, who have provided information relative to their diagnostic services, emphasized the primate and what is available there.

There are a number of you who are aware of what is offered, but I am sure a number of you in the audience might not be aware that there is a diagnostic laboratory for simian virus diseases that is funded and the question of charges always comes up. There aren't any charges here. Animal Resources of NIH provides the funding, with a little bit of support from WHO. It's sometimes stressed the other way around, but I wanted to get that point. It's primarily NIH supported. Support is available for those who have primate colonies who wish to obtain information on diseases that occur in their colonies. There are a number of diseases that do occur, and, at times, there are questions as to how or where such a service for diagnostic capabilities can be obtained. I do wish to indicate at this time that such a service can be provided at the Southwest Foundation for Research and Education.

CHAIRMAN: I have a question for you. Dr. Dolowy mentioned there was a problem of investigators wishing to have work done that was related to their experimental procedures rather than just spontaneous disease. How do you handle that problem?

DR. RICHTER: We've had that problem occur, I think, just as often as anyone else. Usually the way we approach it, is that we look at the first animal or two, just to reassure ourselves that there is no spontaneous disease problem involved. This also helps to convince the investigator that we just haven't turned our back on him and refused to help him. In the process of doing this, we usually can convince him, through discussion of the case, that this is really part of the experimental procedure and not part of the diagnostic problem. So we handle it very much on an individual type of basis, depending on the investigator and what the problem is.

CHAIRMAN: Do you perform routine services for him? You know, if he needs to have some pathology workup done as part of his protocol, will you perform that for him strictly on a service basis?

DR. RICHTER: As a general rule we haven't. There hasn't been too big a demand for this because most of the people, most of our investigators, already have these facilities.

J. L. WAGNER, University of North Carolina, Chapel Hill: If these facilities did not exist, would you then perform them for an investigator?

DR. RICHTER: There are some facilities in the University that on a fee basis will perform them. What we have been afraid of, because we've had so many requests for this kind of thing, is that we would be overwhelmed with

doing routine services, running blood counts or routine histology for people doing experimental work. We usually try to find, and help them find other arrangements. For example, the pathology department operates a training school for histotechnicians; and, on a fee basis, they will cut slides for people. So if after our initial contact, we determine that all they need is a routine laboratory to provide a technical service, we refer them to a lab like this. We've tried to be a clearinghouse by trying to find a satisfactory arrangement without we ourselves getting into a long-range business of providing routine laboratory service.

PROGRESS IN THE DEVELOPMENT OF A HISTOCOMPATIBLE DOG

J. W. Templeton, A. L. Rogers and W. S. Fletcher*

The Departments of Surgery and Animal care at the University of Oregon Medical School (UOMS) maintain a canine breeding colony which produces approximately 300 purebred Labrador retrievers each year for medical research purposes. The colony was established in 1961 for the specific purpose of producing well-defined dogs for medical research with a primary goal of producing a large histocompatible dog. There are several ongoing programs in the colony for better defining the dog as a research animal. These programs are basic husbandry studies, analysis of costs of producing dogs, physiology studies, reproductive physiology and genetic studies.

The basic husbandry studies include optimum methods of whelping, health care, feeding, cleaning, penning, socialization and record keeping. The basic production cost of rearing a dog to six months of age has been determined to be $80.79. The physiology studies have been primarily in the area of cardiac function in the developing dog. The reproductive physiology studies are at present emphasizing artificial insemination and the storing of frozen semen. There have been ten litters born in the colony to date from bitches impregnated with frozen semen stored from one to nine months.

The genetic studies have primarily been concerned with the theoretical possibilities of producing a histocompatible dog as a surgical model for organ transplantation, genetic, immunological, cancer and other studies. The development of a histocompatible dog would allow the maintenance of transplantable tumors and open up a whole new area of investigation in an animal of such size as to allow surgical procedures and of such longevity as to allow prolonged chemotherapeutic and immunologic studies.

INTRODUCTION**

It is the basic premise of the authors that one of the primary problems of medical research performed on dogs is the dog itself. Although scientific institutions have been meticulous about the quality of other animals used in research, they have been content for the most part to use dogs of unknown age, health, temperament, genetic and nutritional backgrounds. The result has been the use of large numbers of dogs for the production of much questionable data. (The National Research Council reported that at least 372,000 dogs were used for research purposes in 1969.[1])

The documentation of the superiority of dogs bred for research to random source dogs is scarce, but it is generally known that there is a high mortality rate of dogs in research for reasons other than the experimental procedures. There is a report from Colorado that a group of random source dogs undergoing renal autotransplants and a routine immunosuppressive schedule suffered a 50% mortality rate for a variety of reasons.[2] The superiority of the Labrador retrievers from the University of Oregon Medical School Canine Breeding Colony to random source pound dogs for experimental heart valve replacement was tested.[3] The dogs selected for experimentation were surplus male Labrador retrievers at least eight months of age or older and weighed 60 pounds or more, and conditioned, healthy pound dogs which weighed 60 pounds or more. There was 79 Labrador retrievers and 55 pound dogs in the experiment (see Table I).

The survival rate was calculated on 5-day postoperative survival and the increased survival rate of the Labrador retrievers to that of the pound dogs is highly significant ($P<0.01$). In addition to altering experimental results the use of pound dogs may also increase research costs in spite of the initial low cost of the dog. In this same experiment the cost of each surgical procedure was $562.06 which does not include the cost of the dog, surgeon's time, overhead, or autopsy costs but only the actual operative and postoperative care expense (see Table II). There is no doubt that these costs

* University of Oregon Medical School, Portland, Oregon 97201.

** This work was supported in part by the National Institutes of Health, PHS Grant No. RR00389, The Louis and Maud Hill Family Foundation, the American Kennel Club, Morris Animal Foundation, and the Dog Fanciers Association of Oregon.

TABLE I.—*Survivors and non-survivors comparing Labrador retrievers and pound dogs undergoing experimental open heart surgery—140 cases; technical errors excluded.*

	Survivors		Non-survivors		Total
Labradors					
Tricuspid	14	(93%)	1	(7%)	15
Mitral	52	(93%)	4	(7%)	56
Rotary aortic	6	(100%)	0		6
Aortic	7	(88%)	1	(12%)	8
Total	79	(93%)**	6	(7%)	85
Pound dogs					
Tricuspid	15	(83%)	3	(17%)	18
Mitral	32	(68%)	15	(32%)	47
Rotary aortic	5	(83%)	1	(17%)	6
Aortic	3	(75%)	1	(25%)	4
Total	55	(73%)**	20	(27%)	75

** Highly significant difference ($P<0.01$).
From: Fletcher et al., Lab. Animal Care 19:506–508, 1969.

have increased so that it is apparent that the cost of the dogs is a minor part of the total expense, yet the untimely death of each subject dog escalates the overall cost of experimentation. It seems conclusive that care must be exercised in selecting dogs used in research and efforts to improve the quality of research dogs should be continued and intensified.

METHODS AND MATERIALS

To obtain a better dog as a research model, personnel from Departments of Surgery and Animal Care began in 1960 to review the origin and development of all known breeds of dogs and selected the Labrador retriever as a breed well suited for inbreeding to develop a strain of large laboratory dogs.[4] In addition to having been inbred while vigorously culled as a sporting dog, Labrador retrievers possess other characteristics desirable in a laboratory dog. These include: large size, short hair, large litters, early maturity, "easy keeping", phenotype uniformity, good cage tolerance, docility, trainability, and stress resistance.

In 1961, the UOMS Departments of Surgery and Animal Care started a breeding colony of purebred Labrador retrievers with 15 dogs all closely related to one outstanding sire, Cork of Oakwood Lane. The breeding plan initially was to alternate inbreeding of one litter with outbreeding as much as possible the next litter. With selection on the original breeders and the addition of new breeding stock, the colony has been enlarged to 40 breeding bitches and 7–8 principal studs. The breeding plan was modified to include two inbred lines composed of 7 bitches and one sire each and an outbred line composed of 26 bitches and 5–6 sires. All 3 Labrador coat colors (black, chocolate and yellow) are present in the colony.

The colony is the subject of a number of ongoing programs of study. They are in the areas of husbandry, physiology, reproductive physiology, and genetics. All of these research projects are designed to define the dog, in order to provide a better research model.

The husbandry program involves the routine management of the mature breeders and approximately 300 puppies per year. The dogs are housed in outdoor runs with a crushed rock bottom. The runs measure 8′ x 40′ or 10′ x 30′. Adult females are housed two per pen and breeding males are housed separately. Three or four dogs of both sexes are housed together up to six months of age. The runs are cleaned daily with a motorized wheelbarrow driven down the aisle between the runs to aid in removing the waste. One full-time employee and one half-time employee are required to clean and rake the gravel in the 122 runs.

TABLE II.—*Costs of one open heart animal experiment*

Blood Donor Dog	$ 34.00	Packs (steam & gas sterilization)	$ 6.00
Three technicians	175.00	I.V. sets	6.86
Recovery room, (five days @ 1.28)	6.40	Gloves, surgical (disposable)	2.80
Room & Board (for 3 months @ 93¢/day)	79.05	Masks (disposable)	1.00
ACD bottles (4)	3.32	Sanborn paper	5.00
D5/W (5) 500 cc	2.40	Tape	.50
D5/W (3) 200 cc	1.23	Calcium Chloride	.75
Penicillin	.50	Suture material	40.00
Isuprel	.70	Scrub sponges	.96
Adrenalin	.30	4 x 4's	1.86
Heparin	3.00	Graft material (teflon felt)	2.00
Pentothal	2.80	Plastic ties	.60
Flaxedil	2.00	Skin towels	.75
Xylocaine	1.00	Surgical blades	1.00
Pump tubing	18.75	Plastic bags	.50
Ringers solution (2) 1000 cc	1.58	Plastic stopcocks	.85
Iodine	.20	P. E. tubing	2.00
Surgical spray	.25	Bicarbonate	1.00
Normal saline (2) 1000 cc	1.00	Bacitracin	1.50
Syringes (disposable)	1.15	Atropine sulfate	.75
Needles (disposable)	.50	Oxycel (cotton)	1.50
O_2	.25	Bardic catheters	10.00
CO_2 in O_2	.50	Total	$562.66

From: Fletcher et al. Lab. Animal Care 19:506–508, 1969.

The dogs learn to use self-waterers in a very short time and are fed ad-libitum, a diet of dried dog food. The food bin, equipped with a weather shield, is filled approximately twice a week. The dogs regulate their food intake well, shown by the fact there have been very few dogs with excessive weight gains. The puppies are offered moistened dry food at three weeks of age and with few exceptions readily adapt to this diet of solid food.

The bitches are whelped in outdoor runs equipped with a house containing a heat lamp. Prior to whelping they are maintained singly. Four by eight foot sheets of plywood are placed against the wire enclosure on each side of the dog house to allow privacy during whelping and reduce the nervousness of the whelping bitch. At birth each puppy is weighed and given an identifying number. A clip mark is used for identifying the puppy until 10 weeks of age and then is replaced by a number tattooed in the ear. A neck chain medallion is also used. The puppies are weighed weekly the first month, every two weeks until 12 weeks of age, and then monthly as long as they are in the colony.

The mild damp climate of Oregon is conducive to external and internal parasites. The endoparasites are controlled by deworming at 3, 5, 7, 8, 12 and 14 weeks of age. Deworming of older puppies and adults is done as required. Ectoparasites are controlled by insecticide spraying of the dogs and the dog houses.

Puppies are vaccinated with a DHL (Distemper, Hepatitis, modified live virus vaccine and Leptospirosis bacteria) at 7, 9 and 12 weeks of age and with rabies modified live virus at 6 months of age. Booster vaccinations of DHL and rabies are given at one and two year intervals, respectively.

The scheduling of worming, vaccinations, weighing, pelvic radiographs, physical examinations, puppy behavioral testing, and blood typing of each dog is done by computer each month. The data collected on each dog are stored in a computer disk file where the information is readily available for analysis.

In 1971 the production cost of raising a dog to 26 weeks of age was calculated to be \$80.79. This included costs for feed, \$31.86; labor, \$43.65; and supplies, \$5.28. The costs will undoubtedly have to be adjusted upward but this figure will always be a minor portion of research costs due to the need for fewer animals required for meaningful data.

Future husbandry research projects which are successful in preliminary studies are indoor whelping, improved individual dog identification by freeze branding, and artificial nursing of neonate puppies which would otherwise have to be put to sleep.

The reproductive physiology research has primarily emphasized artificial insemination and long term preservation of dog semen. The first reported puppies born from a bitch inseminated with semen stored frozen for any length of time was from this colony.

To date there have been 10 successful pregnancies from semen stored frozen from one to nine months. The possibility of preserving semen for wide usage in improving breeds of dogs is imminent. The use of stored semen in maintaining genetic stocks for medical research models is certainly an important aspect of the semen preservation and artificial insemination studies.

Another reproductive physiology study being developed in this colony is the effects of various light spectras on the female's reproductive performance. Female dogs are being reared from twelve weeks of age to two years of age in rooms that have light sources which are commercially available but have very unusual spectral qualities. The light sources are mercury arc vapor, incandescent, warm white fluorescent, and a full spectra fluorescent. The dogs will be followed through puberty and studied for deleterious effects of the light exposure to their breeding capabilities. The effects of these light exposures on various hormones such as lutenizing hormone, progesterone, and estrogens will be studied. The levels of these hormones will be detected by radio-immunoassay and protein binding.

The physiology studies to date have been concerned with defining the oxygen dissociation curves of the growing dog. The P_{50} at birth is 21.2 ± 0.4 mm Hg and it shifts to the right to a maximum of 34.5 ± 0.4 mm Hg at approximately 65 days of age. The P_{50} slowly shifts back to 31.7 ± 0.1 mm Hg at approximately 200

days of age where it stabilizes for the rest of the dog's life. The shift in P_{50} as the dog grows is accomplished without the production of new hemoglobins but is correlated with the levels of diphosphoglycerate.[5]

The use of red cell blood groups in defining dogs in this colony has been developed with the aid of Dr. Scott N. Swisher.[6] Members of the colony are typed for the Swisher canine blood groups A (A_1 and A_2 alleles), B, C and D and the antiserums needed to detect these antigens are all produced in this colony. Current research efforts in the blood group system include efforts to better define the A blood group by absorption and a search for new dog blood groups.

The genetics studies in this colony have been concerned with coat color inheritance, and genetic mechanisms of deleterious traits including hip dysplasia, entropion, and glaucoma.

The main emphasis has been on preliminary studies of the theoretical possibilities of breeding a histocompatible dog. It was considered that the conventional method of full sib matings used to produce histocompatible lines of rodents would be a difficult way to produce histocompatible dogs because of the longer generation time of dogs, the greater chromosome number (Dog 2N = 78), and the amount of space needed to hold enough dogs to buffer the deleterious effects of inbreeding. These reasons also make the full sib inbreeding economically unfeasible for dogs. The breeding plan chosen was to develop *in-vitro* methods for direct selection on histocompatible antigens and to test progeny histogenically by skin allografts.

The method of one-way stimulation by mixed leukocyte culture (MLC) was developed initially for use in the histocompatibility studies because of the contributions MLC has made to the understanding of human transplantation genetics.[7] After the MLC test was established the lymphocyte toxicity test was adapted using four antisera designated A, B, C and D provided by Dr. E. D. Thomas, Seattle, Washington and four antisera designated d, e, F and G provided by Dr. J. W. Ferrebee, Cooperstown, New York. These eight canine anti-lymphocyte isoantisera had proved useful in matching dogs for prolonged allograft survival.[8,9]

The preliminary studies of breeding a histocompatible dog were to determine the genetics of the dog transplantation antigens, to test the efficiency of selecting breeders by *in-vitro* methods that recognize histocompatibility antigens, and to assemble lines of dogs that have the same transplantation antigen phenotype for inbreeding.

The transplantation genetics studies were initially attempted by doing family studies with the eight cytotoxic antisera and by MLC studies that Bach and Amos used to study HL–A genetics in humans.[7] The family studies with the cytotoxic antisera were not very rewarding. The specificities of the antisera used are too broad to collect segregation and linkage data. There will need to be more antisera with narrower specificities produced before the technique of cytotoxicity can be utilized to study the genetics of transplantation antigens. In this regard, two new antisera have been produced in this colony. Littermate dogs which were matched with the eight cytotoxic antisera but were mismatched in MLC were cross-immunized with lymphocytes. Both dogs developed antibodies to each other's lymphocyte antigens (see Table III).

The transplantation genetic studies using MLC were more productive. The results were MLC reactivity between all 296 pairs of unrelated dogs tested and no MLC reactivity be-

TABLE III.—*The lymphocyte antigen phenotype and MLC results of littermate dogs, no. 9222 and no. 9223*

Dog no.	Swisher Blood Group	A	B	C	D	d	E	F	G
9222	C	+	+	−	+	+	+	+	+
9223		+	+	−	+	+	+	+	+

MLC Results

Responding Cells

Stimulating	9222_r	9223_r	X^a_r
9222_s	571 [b]	2373	4938
9223_s	1805	447	2262
X_s	4230	2805	221

[a] Unrelated control dog.

[b] Average of two cultures each

Dogs 9222 and 9223 were cross immunized with 10^8 lymphocytes intravenously three times.

9222 developed 1:40 titered antiserum against 9223's lymphocytes.

9223 developed 1:20 titered antiserum against 9222's lymphocytes.

tween 73 pairs of 268 pairs of sibs tested.[10] It was concluded from these data that the dog had a single major histocompatible locus, termed the DL–A locus, by the following reasoning:

A. The data indicated that antigens responsible for reactivity in MLC are controlled by genes at one chromosomal locus.
B. Reactivity in MLC correlates with the results of matching by certain cytotoxic antisera.[11]
C. These same cytotoxic antisera recognize strong histocompatible antigens.[8,12]
D. Testing by MLC and cytotoxic antisera is useful in matching dogs for transplantation of marrow,[8,13] kidney,[12,14] liver,[15,16] and heart.[17]

The preliminary studies to test various methods of selecting dogs to breed in order to produce DL–A matched littermates showed that the matching of parents by MLC and cytotoxicity was the most efficient (see Table IV). Littermates born of parents matched by both MLC and cytotoxicity were all nonreactive in MLC but littermates that were born of parents matched with cytotoxicity alone were nonreactive 81% of the time. Littermates that were the result of three generations of inbreeding were 60% nonreactive in MLC. Based on these data it was concluded that the planned direct selection on histocompatible antigens should make the breeding of a histocompatible dog successful and reduce the amount of inbreeding normally required.

Due to the promising results of the preliminary study of direct selection on DL–A antigens for producing DL–A matched littermates, it was decided to assemble two lines of dogs for further inbreeding and selection. The two lines are presently composed of breeders with the lymphocyte antigen phenotypes BeFG (Line I) and ACFG (Line II) (see Table V). The lines are composed of 1 sire and 7 bitches which all have the same lymphocyte antigen phenotype and are all nonreactive in MLC. The first litter born in one of these lines is currently 4 months of age. At six months of age the littermates will be tested by cytotoxicity, MLC and histogenically by skin allografting. Selection of future breeder replacement for Lines I and II will be based on the results of this testing.

TABLE IV.—*MLC results of littermates from parents selected by various methods*

Number of Pairs	Relationship	Per cent MLC Identical [a]
314	Unrelated	0
268	Sibs from unrelated parents	27
50	Sibs with inbreeding coefficients of 45%	60
32	Sibs from sero-type identical parents	81
30	Sibs from sero-type and MLC identical parents	100

[a] Indicates a greater degree of histocompatibility.

From: Templeton and Fletcher. Surgical Forum 22:263–264, 1971.

TABLE V.—*Lines I and II DL–A and Swisher Red Cell Antigen Phenotypes*

Line I (Chocolate Coat Color)			Line II (Black Coat Color)		
Male	DL–A	Swisher Blood Groups	Male	DL–A	Swisher Blood Groups
1004	BeFG	A_1C	5215	ACFG	A_1C
Females			Females		
7229	BeFG	A_1C	5391	ACFG	A_1C
9665	BeFG	A_1C	5394	ACFG	A_1C
10050	BeFG	A_1C	9571	ACFG	A_1C
9662	BeFG	A_2C	9689	ACFG	A_1C
7125	BeFG	A_1C	10264	ACFG	A_1C
10241	BeFG	A_1C	10265	ACFG	A_1C
10242	BeFG	A_1C	10266	ACFG	A_1C

SUMMARY

One-way mixed leukocyte culture (MLC) and ten canine antisera have been utilized for selecting dogs for further inbreeding and organ allografting. The application of these techniques in this colony has given the following results:

1. The determination of a single major histocompatible locus in dogs (DL–A).
2. The matching of dogs for breeding with litters being produced that are all matched at the DL–A locus.
3. A program to produce new lymphocyte isoantiserums has resulted in the production of two new antiserums which appear to have narrow specificities.

REFERENCES

1. YAGER, R. H. Summary of questionnaire from National Research Council on number of animals used for research in 1969. Letter dated October 22, 1970.
2. STARZL, T. E., BRETTSCHNEIDER, L., MARTIN, A. J., GROTH, C. G., BLANCHARD, N., SMITH, G. V., and PENN, I. Organ transplantation, past and present.

The Surgical Clinics of North America 48 (No. 4): 817–838, 1968.

3. Fletcher, W. S., Herr, R. H., and Rogers, A. L. Survival of purebred Labrador retrievers versus pound dogs undergoing experimental heart valve replacement. Lab. Animal Care 19:506–508, 1969.
4. Fletcher, W. S., Rogers, A. L., and Erickson, L. F. The need for a large standard laboratory dog. Lab. Animal Care 16:1–8, 1966.
5. Dhinsda, D. S., Hoversland, A. S., and Templeton, J. W. Postnatal changes in oxygen affinity and concentrations of 2,3–diphosphoglycerate in dog blood. Biology of the Neonate (in press), 1972.
6. Swisher, S. N., and Young, L. W. The blood grouping systems of dogs. Physiological Reviews 41:495–520, 1961.
7. Bach, F. H., and Amos, D. B. Hu–1: Major histocompatibility locus in man. Science 156 (3781): 1506–1508, 1967.
8. Epstein, R., Storb, R., Ragde, H., Thomas, E. D. Cytotoxic typing antisera for marrow grafting in littermate dogs. Transplantation 6 (No. 1):45–58, 1968.
9. Mollen, N., St. John, D., Cannon, F. D., and Ferrebee, J. W. Lymphocyte typing in allografted beagles. Transplantation 6:939, 1968.
10. Templeton, J. W., and Thomas, E. D. Evidence for a major histocompatibility locus in the dog. Transplantation 11:429–430, 1971.
11. Rudolph, R. H., Hered, B., Epstein, R. B., and Thomas, E. D. Canine mixed leucocyte reactivity and transplantation antigens. Transplantation 8 (No. 2):141–146, 1969.
12. Rappaport, F. T., Hanaoka, T., Shimada, T., Cannon, F. D., and Ferrebee, J. W. Histocompatibility studies in a closely bred colony of dogs. J. of Exper. Med. 131:881–893, 1970.
13. Storb, R., Epstein, R. B., Rudolph, R. H., Thomas, E. D. The effect of prior transfusion on marrow grafts between histocompatible canine siblings. J. Immunology 105:627–633, 1970.
14. Kisken, W. A., and Malek, G. H. Kidney allograft survival in the dog predicted by mixed leucocyte culture tests. Nature 224:1010–1011, 1969.
15. Marchioro, T. L., Haugie, C., Ragde, H., Epstein, R. B., and Thomas, E. E. Organ homografts for hemophilia. Transplantation Proceedings 1:316–320, 1969.
16. Chandler, J. G., Viillar, H., Lee, S., Williams, R., Ferrebee, J. W., and Orloff, M. J. Orthotopic liver transplantation in inbred dogs matched according to lymphocyte types. Surgical Forum 21: 343–345, 1970.
17. Boyd, A. D., Ferrebee, J. W., Cannon, F. D., Gherunpong, C., Lower, R. R., Spencer, F. C., and Rappaport, F. T. Prolonged survival of cardiac allografts in a closely-bred dog colony. Surgical Forum 21:188–190, 1970.
18. Templeton, J. W. and Fletcher, W. S. The development of a large histocompatible dog. Surgical Forum 22:263–264, 1971.

DISCUSSION

A. J. Wilson, Ohio State University, Columbus: Did I understand you correctly, one animal technician takes care of about 400 animals?

Dr. Templeton: No. There are 122 runs and 1.5 full-time employees are needed for cleaning them only. It takes two full-time caretakers to feed, clean, and do some research. It is difficult to really designate what is not research currently because we are doing research with the same personnel. As for the cost of these dogs, there's a fellow here who breeds beagles commercially. I think he could address himself more clearly to the costs of raising a dog, especially a beagle, to six months of age if anyone really wants to pursue that. But I feel that $150 for a dog like this comes pretty close, if you have the right kind of capital investments and include all the top personnel and other expenses into raising one of these dogs to six months of age.

M. L. Povar, Brown University, Providence, R. I.: Can you tell us a little about your experience with hip dysplasia?

Dr. Templeton: Hip dysplasia is endemic in all large breeds and we do have a hip dysplasia study. We can just say we have a lot of it. We have calculated hereditability on this, and it has a hereditability estimate of around 0.35, 0.4, or something on that order. I think that we have reduced the instance by selection, much as they've done in the Swedish colony of German Shepherds or the Swedish Army dog colonies. It is a problem in all large breeds, and there are some breeds in which all members are dysplastic as you know. Every dog is radiographed at six months of age and it is repeated every six months as long as the dog is available to the colony. That is one of the criteria for selecting breeders.

H. P. Schneider, Hahnemann Medical College, Philadelphia: Would you include the greyhounds when you say that hip dysplasia is a problem in all large breeds?

Dr. Templeton: This is a very confusing subject. I have seen one radiograph alleged to be

of a greyhound that was dysplastic. However, there is such a wonderful selection against hip dysplasia in the greyhound. They are a very biased breed, because if they don't win at the stakes, they're put on the table. They are not frequently bred as pets. Therefore, any greyhound with the slightest trace of these genes have no opportunity to breed. But it is there, I'm sure. I have heard rumors from other people, but I'd say any breed in which dogs grow to weigh around forty pounds or more will have it. And those that get larger, have it worse. We have shown quite clearly in our colony that the more rapidly growing, robust dogs have the worst hips.

C. STORMONT, School of Veterinary Medicine, Davis, California: I think this is a very good project like you said, Dr. Templeton, but the objective of selection is for adaptability. It seems to me this has it all over these long-term in-breeding projects. I know some that are and have been going on and have cost millions of dollars, and they're not getting any place. You have an objective. You can select your histocompatibility and once you've got it reasonably well fixed in your dog colony, this should be an excellent dog for all kinds of transplantation procedures.

DR. TEMPLETON: I think that if you need uniform dogs they will be supplied by this breeding plan and you can also provide dogs defined in the genetics of histocompatibility.

THE PRIMATE INFORMATION CENTER AS A RESEARCH TOOL IN STUDIES OF THE HEART AND LUNG

Maryeva W. Terry*

The Primate Information Center can assist with literature research for projects involving nonhuman primates in several ways. It can provide published norms on many variables, determine if there are previous related studies, or provide citations on fundamental structure and function. Such searches can be restricted to a single genus or species of nonhuman primate or can cover all genera.

INTRODUCTION

Beyond biological considerations, a unique advantage to use of non-human primates as subjects of biomedical studies is availability of a literature search service dealing exclusively with these animals. The Primate Information Center is prepared to expedite literature research in several ways.

It can provide data on normal cardiovascular, respiratory, hematologic and blood chemical variables in any species of nonhuman primate that has been studied in this regard. A number of tabulations of variables relevant to cardiovascular research are available (see below). In addition abstracts on such variables as heart rate, respiratory rate and minute volume are on file and can be duplicated in response to specific requests.

It can determine whether there has been previous attempts to develop a model of a clinical syndrome in nonhuman primates. If such efforts have been made, the Information Center will provide the relevant citations, regardless of whether the attempt was successful or unsuccessful. Conversely, it can provide citations on effects of specific experimental manipulations.

It can provide bibliographies for any or all species of nonhuman primate on the basic structure and function of the cardiovascular and respiratory systems, spontaneous occurrence of diseases also seen in man, transplantation studies, pharmacologic studies, or research methodology.

It can provide recurrent service on any bibliography, so that new citations on the original search topic will be forwarded as soon as they are processed.

It can provide a weekly listing of recent citations on all areas of primate research and the addresses of the authors, so that reprints may be requested. This service is called *Current Primate References.*

HOW TO OBTAIN SERVICE

All these services are available without charge. Operation of the Information Center is wholely subsidized by the Regional Primate Research Center, which is supported by grant RR00166 from the Animal Resources Branch of the National Institutes of Health. The user need only contact the Primate Information Center, Regional Primate Research Center SJ-50 of the University of Washington, Seattle, Washington 98195.

Normal Values

The papers listed below may be obtained alone or with supplemental abstracts related to a given primate species. Abstracts on other variables may be obtained by specifying the variable; restriction of the request to a particular species is not necessary, but will be honored. Owing to the volume of material in these files, requests for all normal values, even if restricted to a particular species, cannot be honored.

The available tabulations, covering all nonhuman primate species for which data were available, and the dates of their preparation are:

Cholesterol and Other Plasma Lipids. 1972

* Regional Primate Research Center at the University of Washington, Seattle, Washington.

Urea nitrogen, uric acid and creatinine in the blood. 1972

Hematologic Values

I. Erythrocytes, Hemoglobin, Hematocrit and Related Indexes. 1970

II. Total Leucocytes and Differential Counts. 1970

Adrenal and Gonadal Hormones in Plasma and Urine. 1968

Enzymes in Blood

I. Cholinesterase, Alkaline Phosphatase and Other Hydrolyzing Enzymes. 1968

II. Serum Glutamic Oxalacetic Transaminase and Glutamic Pyruvic Transaminase. 1968

III. Oxidoreductases Including Dehydrogenases. 1968

Literature Search

For best service, a letter requesting search for possible citations should contain the subject of the desired search, stated as one might state the title of an article, and a brief statement of the research problem. Searches are performed by specialists who are routinely analyzing literature in the subject area. They are sometimes able to suggest supplemental literature of probable interest or to tailor the response more closely to the specific need if they have some idea of the use of the response.

In general, the initial response will be prepared by searching indexed bibliographic files covering literature published since 1965. The Information Center has additional files covering literature published between 1940 and 1964. If no relevant citations are found in the recent literature, this collection of older citations will be searched automatically; otherwise, search of the literature published before 1965 must be specifically requested.

Finally, any subject search can be restricted to a particular genus or species of nonhuman primate, but the Information Center does not undertake to prepare comprehensive bibliographies of a genus or species. In this regard, it should be noted that the files of the Information Center cover all topics in primate research, not just those related to biomedical research, so that a comprehensive bibliography of a species would contain a high percentage of citations of no interest to an individual user.

CHAPTER XIII

MONITORING

Gamma-Ray Densitometry in the Analysis of Hemodynamic Function

The Use of a General-Purpose Time-Shared Computer in Physiology Research

Biotelemetry Transmitter Design Methods

Measurement and Radiotelemetry of Cardiovascular Variables in Conscious Animals: Techniques and Applications

Implant Telemetry and Stimulation

Capabilities of Implantable Transducers

A Mathematical Model for Measuring Blood Flow By Residue Detection When Radiotracer Recirculation Interferes

Pulsed Doppler Ultrasonic Flowmetry

Implantable Ultrasonic Blood Flowmeters

Percutaneous Conduction of Electrical Signals: Interface Techniques and Tissue Reaction at Electrode Site

The Use of Implantable Telemetry Systems for Animal Monitoring

The Adaptive Control of Therapeutic Procedures

Real-Time, Time-Sharing Computers in the Animal Laboratory

Review of Animal Instrumentation: Transducer Capability

A Multi-Station Computer System for Acquiring and Processing Animal Data

The Conscious Calf Preparation: Technical Aspects of Surgery, Animal Care, Implanted Instrumentation and Monitoring for Cardiovascular Data Acquisition

GAMMA-RAY DENSITOMETRY IN THE ANALYSIS OF HEMODYNAMIC FUNCTION

J. D. Cohn, K. Ito and L. R. M. Del Guercio*

An instrument has been developed to record contrast dilution curves by a noninvasive technique. Transthoracic roentgen density alterations are recorded by use of a solid state radiation detector array. A small amount of injected non-radioactive, radiopaque contrast material is distributed throughout the central circulation. The detector array, positioned over the cardiopulmonary silhouette, records changes in density during the passage of the contrast material, and typical indicator dilution curves are transcribed. An experimental model was constructed to verify the use of the contrast dilution analyzer system in the analysis of transit time functions. In animal studies, pulmonary circulation time was computed from contrast dilution curves obtained across the central circulation. The pulmonary circulation time in normal, anesthetized dogs was found to be 3.13 seconds.

INTRODUCTION

Indicator dilution studies are currently employed clinically as an adjunct in the analysis of hemodynamic function. In practice, indocyanine green dye is injected into the central circulation and optical density changes are continuously recorded from sampled arterial blood. The transcribed indicator dilution curves allow calculation of total blood flow, transit times and vascular volume distribution.[1] Quantitation of these hemodynamic parameters and the qualitative description of the shape of the indicator dilution curve allow assessment of hemodynamic function and document the presence of intravascular shunting phenomena.

Contrast dilution analysis through use of gamma-ray densitometry allows analysis of hemodynamic function within the confines of the central circulation and without the use of invasive catheter techniques. Campeti and Palladoro[2] recognized the analogy between density changes on cineangiograms and dye dilution curves. The cinedensigrams they published were similar in form to indicator dilution curves and demonstrated the effects of intrathoracic shunting on the recorded roentgen contrast dilution curves. Lind, Spencer and Wegelius[3] likewise noted the relationship between serial angiographic density changes and congenital cardiac defects. Abnormal contrast dilution curves were visualized on recordings taken over individual cardiac chambers and vascular segments.

Roentgen contrast recording techniques have been effectively developed and are utilized in research. Williams and associates[4] obtained indicator dilution curves by roentgen video densitometry following intracardiac injection of a radiopaque contrast agent. The dilution curves are undistorted by catheter sampling systems and areas circumscribed by the pulmonary or cardiac silhouette may be investigated with ease.

In an attempt to further develop a method for the assessment of hemodynamic function based on analysis of indicator dilution curves, Del Guercio and co-workers[5] and Cohn et al.[6] utilized a transducer to record roentgen density changes during contrast medium transport. This device consisted of a photomultiplier tube covered by a small fluorescent screen. The collimated x-ray beam passed through the subject causing the screen to fluoresce, thus exciting the photomultiplier cell. Absorption of the x-ray beam energy by the contrast material is reflected in the photomultiplier tube output voltage and recording of contrast dilution curves are readily obtained over areas inaccessible to catheterization techniques. By this technique, roentgen contrast dilution curves were serially recorded over the lung parenchyma during the onset of hemorrhagic shock. Alterations in the recorded contrast dilution curves reflected

* Surgical Research Laboratory, Saint Barnabas Medical Center, Livingston, New Jersey.

changes in blood flow distribution and transit times.[7]

Further refinement in methodology has led to the recent development of a sensitive solid state array system for recording simultaneous indicator dilution curves over the entire cardiopulmonary silhouette. The potential for this mode of hemodynamic analysis is great. Through the injection of a small amount of non-radioactive, radiopaque agent, circulatory parameters are recorded over multiple areas of the heart and lung. By this means, intrathoracic shunts are detected, blood flow distribution may be defined and ejection fraction evaluated.

INSTRUMENTATION

The roentgen densitometer unit* consists of an x-ray or gamma-ray source and a detector array attached to a mobile support structure (Figure 1). The detector array is mounted on a movable support unit under the detector housing. By this means, detector position in relation to the experimental subject may be remotely controlled by use of electronic circuitry located on the console panel. The sensor device consists of solid state radiation detectors with the detector area measuring 20 square mm. The diameter of the detector housing structure is 2.5 cm. Hence, multiple detectors may be placed within the confines of the cardiopulmonary silhouette. Currently a linear array of six identical detectors is utilized and six simultaneous contrast dilution curves are thus recorded.

The radiation detector device operates at room temperature. Efficiency with x-ray energies of 60 kv is approximately 10 percent and the efficiency increases with decreasing x-ray energies. Output signals from the solid state detector are processed through pre-amplifiers and amplifiers and count rates are noted on rate meters connected to each output channel. Filter modules are incorporated into each channel for optimal signal recordings. All curves are recorded simultaneously on a strip chart recorder.

Calibration is achieved by recording transthoracic density changes during the passage of step-density wedges between the source and detector sites. Calibration curves obtained in this manner appear nearly linear within the ranges of x-ray voltage employed in these studies. Simultaneous recording of six contrast dilution curves is performed by positioning the detector array over the cardiopulmonary silhouette. Precise anatomic position control is not

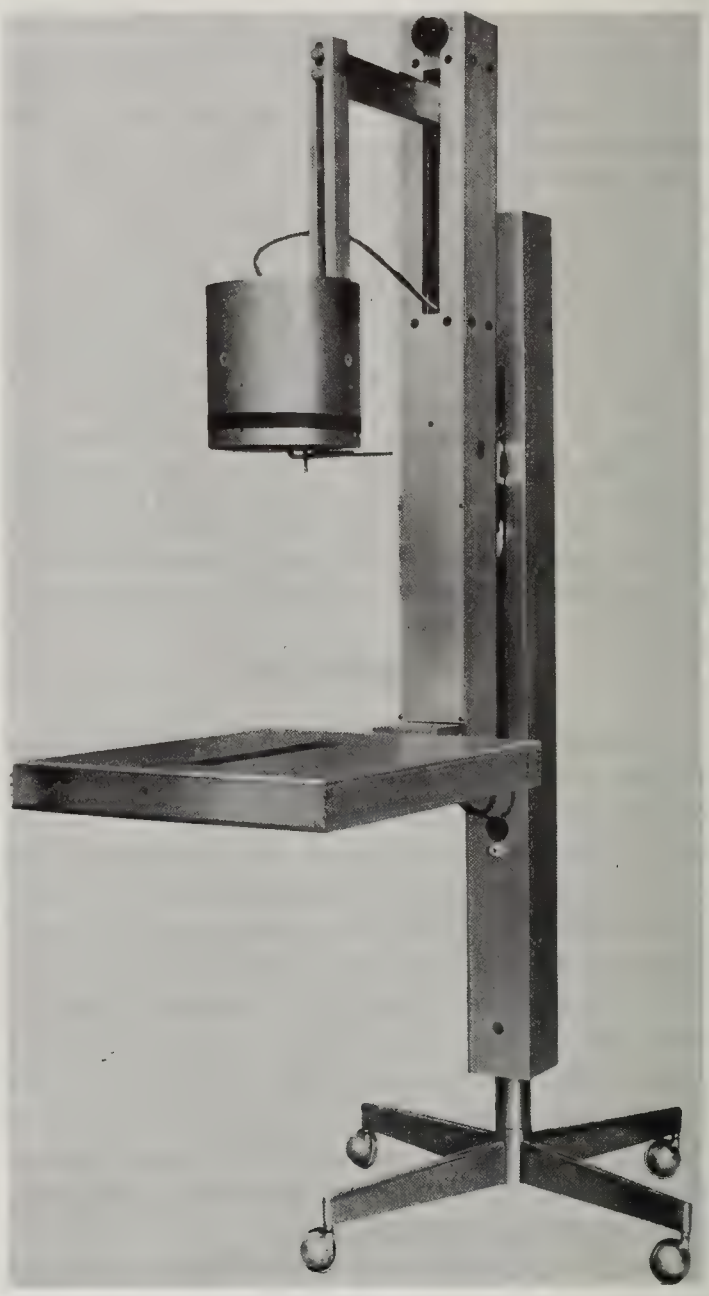


FIGURE 1.—The Contrast Dilution Analyzer System consists of an x-ray or gamma-ray source and a detector unit. The source head assembly shown above houses a 3 curie americium–241 source. Solid state radiation detector devices are housed in the base of the unit and their position is controlled through the instrument console. The subject is placed between the isotope source and detector unit and multiple, simultaneous contrast dilution curves are obtained.

* Contrast Dilution Analyzer, Physiologic Interface Corporation, Box 211, King of Prussia, Pa. 19406

required since qualitative analysis of the recorded curves allows correlation with physiologic events. Following positioning, as above, amplifier gain is adjusted and 5 ml meglumine diatrizoate is injected into the central circulation and the injection time noted. Contrast dilution curves are then transcribed on the six-channel strip chart recorder. These indicator curves, then, reflect the changes in transit times related to the passage of blood through the pulmonary circulation and cardiac chambers.

EXPERIMENTAL MODEL

In order to evaluate this system for the analysis of transit time measurements, a model flow circuit was constructed employing circulation through an open loop, constant flow system. A flexible plastic tube was fashioned into a loop and connected to a water tap. Constant water flow was maintained through the open-ended tube. A single solid state radiation detector was positioned under the plastic tube at the point where the loop crosses itself. The radiation source was then positioned in line with the detector and plastic tubes. By this means the mean particle transit time through the loop configuration is given by the equation,

$$t = L\tfrac{1}{4}\,\pi\, d^2/F \qquad (1)$$

Where t = transit time (sec)
L = length of tubing loop (cm)
d = tubing diameter (cm)
F = fluid flow (cm^3/sec)

Contrast dilution curves were recorded through use of this system following the upstream bolus injection of 5 ml meglumine diatrizoate. Indicator dilution curves were transcribed on each passage of contrast agent through the field of the solid state radiation detector. As the contrast agent circulates through the tubing loop dispersion occurs and is reflected in the curve recording on the second passage through the field of the radiation detector.

From the recorded contrast dilution curves and their respective calibrations, transit time functions are computed by numerical analysis.[1] The algebraic difference between the mean transit time related to each passage of contrast agent through the field of the radiation detector represents the transit time through the loop system and should be identical to the transit time derived from equation (1). Figure 2 illustrates the results of this experiment. A plot is shown relating the calculated mean transit time to the mean transit time based upon computations derived from analysis of the contrast dilution curves. The abscissa represents transit time functions obtained through use of equation (1) and the ordinate relates the numerical transit time based upon the roentgen dilution curves. Excellent agreement is noted along the line of identity for all points and the correlation coefficient for the data is 0.9980.

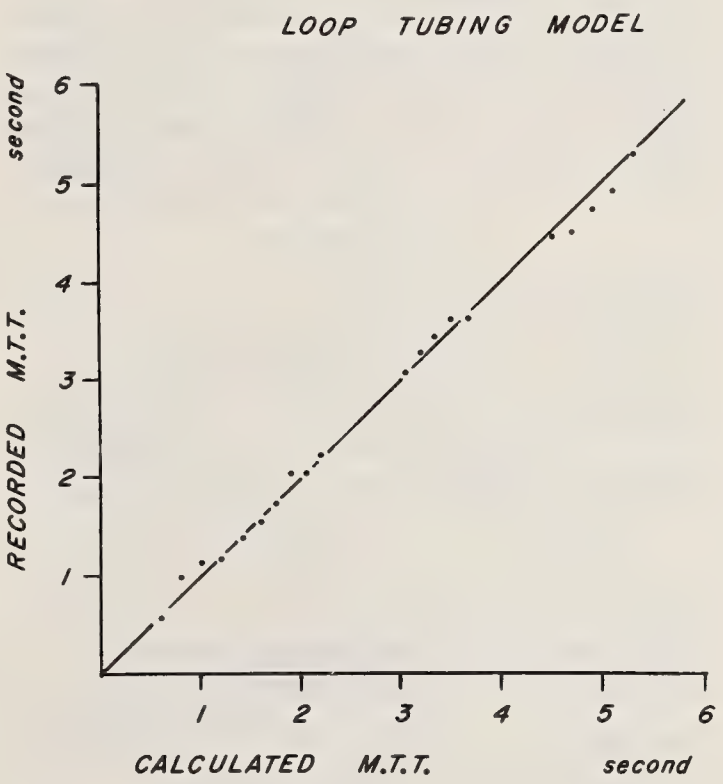


FIGURE 2.—Computation of transit time based on a loop circulation model is shown in the above illustration. Calculated mean transit time (abscissa) is computed from fluid flow and tube dimensions according to equation (1) and recorded mean transit time (ordinate) is calculated solely on the basis of the shape of the recorded contrast dilution curves. The line of identity extending from the origin is illustrated. The least-squares fit of the above data is given by the equation, $y = 0.9567 \times +0.1156$ and the correlation coefficient for the paired data is 0.9980.

ANIMAL STUDIES

Animal studies were performed with adult, 15–20 kg mongrel dogs. Anesthesia was instituted with the intravenous administration of sodium pentobarbital. Following artificial ventilation and hemodynamic stabilization serial recordings of contrast dilution curves were obtained in a standard fashion.

Contrast dilution curve recording was obtained by positioning the six-channel solid state detector array across the central circulation. Gain and position circuits were adjusted and apnea then induced for the duration of curve inscription. Three to five ml. meglumine diatrizoate were then injected into the central venous circulation, and contrast dilution curves were recorded as shown in Figure 3. Immediately following curve transcription, step density wedges were placed within the roentgen beam path to allow calibration of the indicator dilution curve signal. Figure 3 illustrates an example of a contrast dilution curve recorded over the aorta and reflects stepwise washout of indicator from the left ventricle. Replotting the step decays on a logarithm scale demonstrates an exponential washout pattern related to each ventricular ejection. Calibration is nearly linear and is employed in replotting the observed logarithm scale.

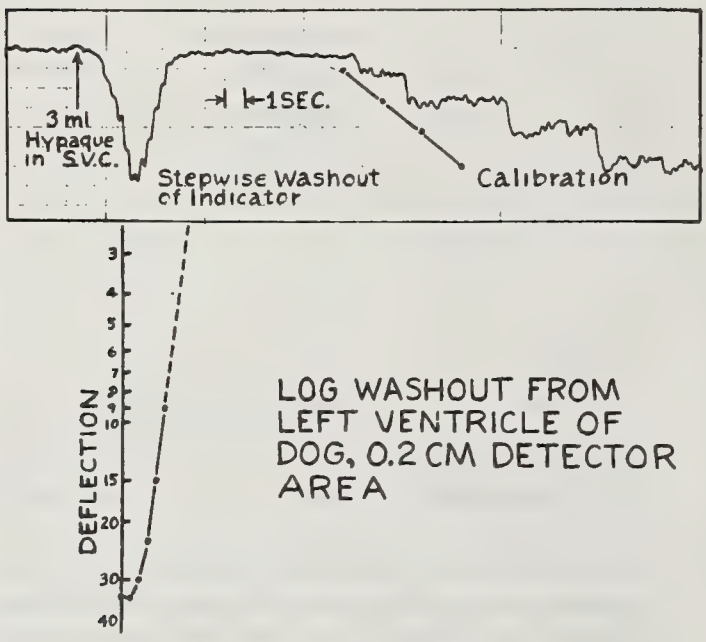


FIGURE 3.—Stepwise indicator washout from the left ventricle is illustrated in the recording of a contrast dilution curve obtained over the aorta. The calibration signal is shown on the right. Replotting of the stepwise washout on the logarithm scale demonstrates an exponential decrement in indicator from the left ventricle related to each ventricular ejection.

Pulmonary circulation time is a physiologic variable useful in the assessment of respiratory and hemodynamic function and can be estimated as the difference in indicator transit times computed from the right and left sides of the central circulation. For estimating pulmonary circulation time, contrast dilution curves were recorded from the central circulation according to the method described above. Recorded curves reflecting indicator dispersion from the right and left sides of the circulation, as shown in the insert in Figure 4, were utilized in the computation of transit time across the pulmonary vascular bed. The recorded contrast dilution curves were smoothed and then replotted onto a logarithm scale. Washout of indicator from both sides of the circulation was assumed to be exponential and all curves were reconstructed to allow computation of mean transit time related to the right and left sided contrast dilution curves as shown in Figure 4.

Data obtained from six normal, anesthetized mongrel dogs relating to the calculation of pulmonary circulation time is shown in Table I. The mean transit time to the right side of the circulation is represented by MTT_1. Point A represents the recomputed appearance time for the left sided curve and the mean transit time to a point in the left circulation is represented by MTT_2. Pulmonary circulation time, MTTp, is then given by the equation,

$$MTTp = A + MTT_2 - MTT_1 \qquad (2)$$

Pulmonary circulation times were obtained in six normal dogs through the analysis of roent-

TABLE I.—*Calculation of Pulmonary Circulation Time*

Expt.	MTT_1 (sec)	A (sec)	MTT_2 (sec)	MTT_p (sec)
1	1.35	2.00	2.80	3.45
2	1.55	2.20	2.10	2.75
3	1.15	1.40	2.90	3.15
4	1.50	2.00	2.10	2.60
5	1.50	2.30	3.30	4.10
6	1.52	1.60	2.67	2.75
				mean = 3.13
				S.D. = ±0.57

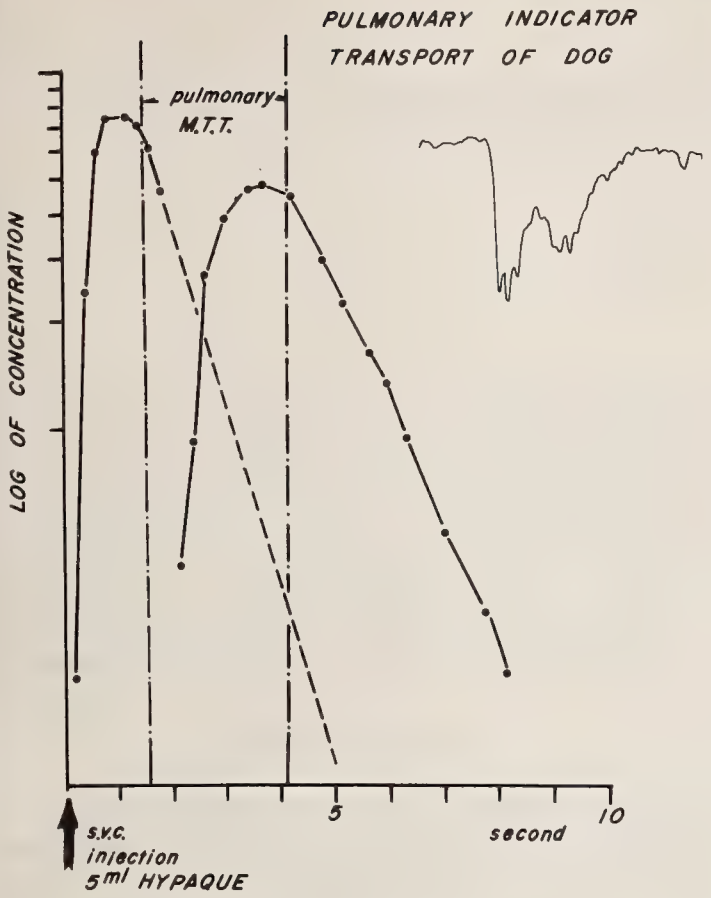


FIGURE 4.—Analysis of contrast dilution curves for calculation of pulmonary circulation time is illustrated above. The original contrast dilution curve is shown in the insert. Curve smoothing is performed and the data is then replotted, by use of the calibration wedges, onto a logarithm scale. Each curve is reconstructed for exponential indicator washout and an appearance time is identified for the left sided component of the contrast dilution curve. In this manner data are obtained for calculation of pulmonary circulation time, as shown in Table I, through use of equation (2).

gen contrast dilution curves and averaged 3.13 ± 0.57 (S.D.) seconds.

SUMMARY

The ability to record multiple contrast dilution curves in an array pattern over the cardiopulmonary silhouette allows for detailed evaluation of hemodynamic function. This newly developed technique employs a single injection of a small amount of non-radioactive, radiopaque contract agent. The small dose injected has no effect on cardiac function. The noninvasive recording system faithfully transcribes changes in blood flow distribution, vascular volume and stepwise washout from the ventricular chambers. This allows analysis of hemodynamic and circulatory phenomena related to cardiac function, intravascular shunting and cardiac ejection fraction.

An experimental model was utilized to evaluate the contrast dilution analyzer system in the analysis of transit time functions. Excellent correlation was noted between expected mean particle transit time and mean transit time derived from the contrast dilution curve data.

Pulmonary circulation time is an important adjunct in the analysis of respiratory and hemodynamic function. This variable was ascertained in intact mongrel dogs through analysis of contrast dilution curve recordings. Pulmonary circulation time in six normal, intact, anesthetized dogs averaged 3.13 ± 0.57 (S.D.) seconds.

REFERENCES

1. HOFFMAN, J. I. E. Calculation of output, central volume and variance from indicator dilution curves. J. Appl. Physiology 15:535–8, 1960.
2. CAMPETI, F. L., and PALLADORO, V. A. Cinecardiometry and cine-electro-kymography. Am. J. Roentgen. 81:778–87, 1959.
3. LIND, J., SPENCER, R. and WEGELIUS, C. Differential analysis of opacification in angiocardiography. Circulation 11:609–14, 1955.
4. WILLIAMS, J. C. P., VANDENBERG, R. A., O'DONOVAN, T. P. B., STURM, R. E., and WOOD, E. H. Roentgen video densitometer study of mitral valve closure during atrial fibrillation. J. Appl. Physiology 24:217–24, 1968.
5. DEL GUERCIO, L. R. M., GOETZ, R. H., and FAWCETT, H. N. Roentgen desitometric dilution curves. Proc. Conf. Eng. in Med. and Bio. 5:56–7, 1963.
6. COHN, J. D., HOLDEN, F. M., and MARKO, A. R. Roentgen densitometry: A method for evaluating cardiopulmonary dynamics. Aerospace Med. 40:217, 1969.
7. COHN, J. D., and HOLDEN, F. M. Roentgen dilution curve analysis of pulmonary blood flow distribution during hemorrhage. Inves. Radiology 7:67–75, 1972.

THE USE OF A GENERAL-PURPOSE TIME-SHARED COMPUTER IN PHYSIOLOGY RESEARCH

E. Dong, G. C. M. Wiederhold*

Digital computers have been used in many situations. This paper describes the use of a general-purpose, time-shared digital computer at Stanford University for physiological research. The functions utilized were: (1) experimental control, (2) on-line plotting, (3) long-term data storage, (4) data reduction, (5) interactive language, (6) mathematical modeling, and (7) simulation. The use of this system has permitted further refinements in the study of vagal-heart rate control in the dog.

INTRODUCTION

In the past ten years there has been a great proliferation of computer services for the researcher in the laboratory. In our University environment where there are multiple computing tasks going on simultaneously, it has been advantageous to have a large general-purpose, time-shared computer with programming in a high level interactive language, in that the researcher is able to complete experiments in a short time between conception and acquisition of the data.

Experiments can be divided into several phases: (1) experimental control, (2) data collection and reduction, (3) long-term storage, and (4) building of mathematical models. Many of these computers can provide valuable assistance. We hope to demonstrate that the availability of this type of computer system has accelerated the development of certain new concepts in the control of heart rate.

At Stanford various types of computer assistance are provided by the facility termed ACME (Advanced Computer for Medical Research).[1,2] ACME is a project sponsored by the Special Resources Division of the National Institutes of Health. It provides time-sharing computer services to the researcher in his laboratory using an interactive programming language termed PL–ACME, which is a sub-set based upon IBM–PL–1 for reasons of compatibility.[3] The machine (Figure 1) has a very large dynamically-shared memory capability to allow easy implementation of algorithms, a permanent centrally maintained file system for reliable and economic long-term storage,[4] and the capability to use alphabetic text as well as numeric data. An important feature to physiological researchers is the direct analog data input and output through a peripheral 1800 computer. There is also direct support for plotting on-line in the laboratory as well as for

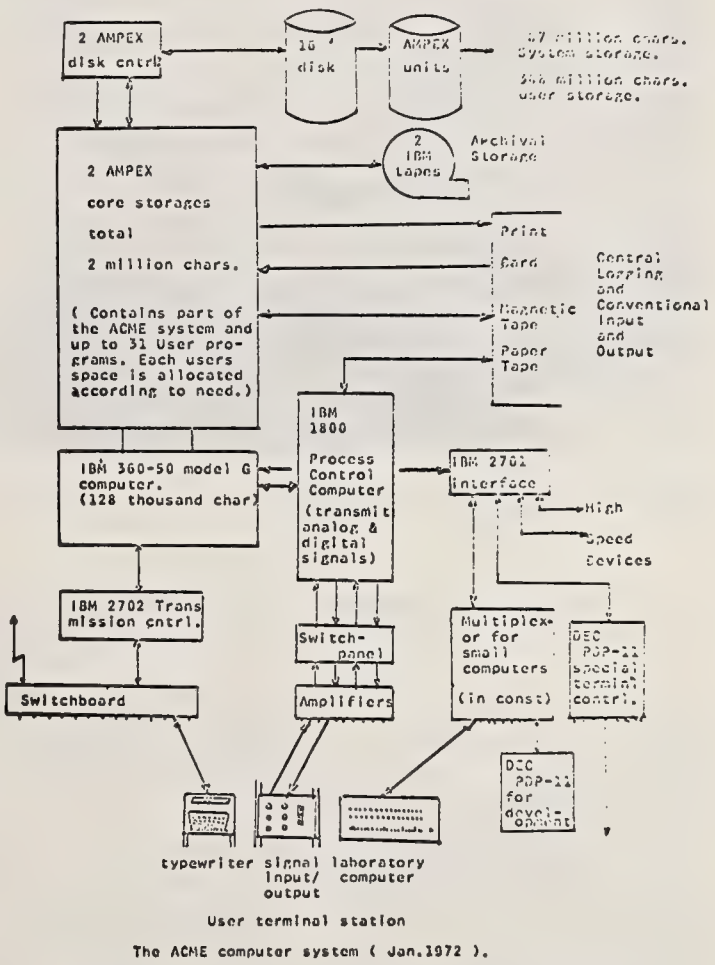


FIGURE 1.—General design of the ACME system.

* Cardiovascular Surgery Division, Stanford University Medical Center, Stanford, California.

cathode ray tube outputs.[5] These facilities are used for a number of projects simultaneously.[6] In addition, the system has made it easy for us to have a stream of ideas which can be tested in series expeditiously. One does not get tied up in the generation of new hardware configurations, nor in the retraining of programmers as the experiments vary and progress (Figure 2).

Table I lists the major projects carried out as part of heart research by the Division of Cardiovascular Surgery at Stanford and shows the features of the system which were required.

The bulk of this presentation consists of a description of the steps used by our group in studying the heart rate response to vagal stimulation and the role this system played in the development and understanding of these concepts. In particular, we were interested in determining a mathematical theory which relates the arterial pressure to heart rate for possible use in a controller for an artificial heart device.

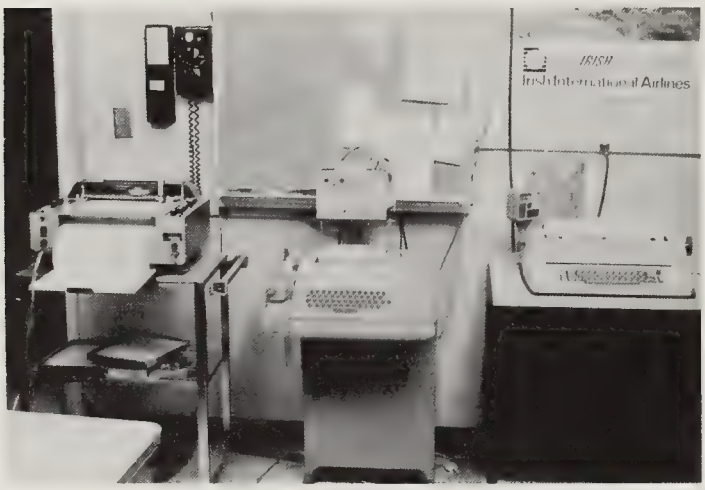


FIGURE 2.—Laboratory equipment and computer interface.

We reviewed the theory on vagal heart rate control. The generally accepted hypothesis that physiologists have taught for a considerable period of time is based on the concept that stimulation of the vagus nerve results in release of acetylcholine, which, by causing an increase in permeability of the cell membrane of the pacemaker, results in inhibition of the heart rate. The heart rate is assumed to be proportional to the concentration of acetylcholine. This is summarized by Homer Warner and his colleagues.[7] We set out to confirm this formulation.

EXPERIMENT CONTROL

The design of the experiment was to evaluate first the effect of vagal stimulation on heart rate, followed by the effect of sympathetic stimulation, and then to develop our own experiments relating heart rate to arterial pressure in an open loop test model.

We used the normal dog anesthetized with morphine, chloralose and urethane, and premedicated with propranolol to block sympathetic activity. We then carried out bilateral vagal transections, and stimulated the cardiac branch of the vagus nerve with a Grass stimulator. The timing and the number of the stimuli were determined by digital computer.

The appendix provides a listing of a program used, and we will make reference to the line numbers of the program where pertinent. This program was written by one of us (E.D.) and expanded by Mrs. Margaret Miller. Several programs and program revisions were used and the one chosen may be considered typical. An array of values were generated based on param-

TABLE I.—*Cardiac Surgery Utilization of the Acme Computer System*

Project	Facilities used							Year
	On-line interaction	Large memory available	Long term files	Alphabetic data	Analog input and output	On-line graphic output	Links to small computers	
Bloodvolume calc.	X							1966
Tabular results		X	X	X				1967
Physiologic data-reduction		X			X			1967
Storage of data from PDP-8			X				X	1967
Graphical analysis of M-waves	X		X			X		1968
Vagus nerve control	X				X	X		1969
Interactive modelling	X	X				X		1970
Patient data files			X	X				1970
M-wave data analysis	X		X		X	X		1971
Ultrasound data processing	X	X	X				X	1972

eters entered by keyboard in the laboratory (program lines 41 to 45) containing zeroes or one-volt values which represented the digital to analog output buffer (program lines 25 to 30). This array is transferred to (line 31) the 1800, and clocked out at 2 msec intervals after receipt of a trigger.

The first protocol that we carried out was a simple repetitive stimulation of the vagus nerve. The stimulation was carried out for 2 minutes. The stimulation consists of a train of bursts. Each burst consists of 1 to 5 stimuli each separated by 10 msec. The period between bursts varied from 250 to 2,000 msec. Figure 3 summarizes the data from two dogs. The horizontal axis gives the stimulus period and the vertical axis is the mean heart period. Each curve has a different number of pulses in the burst. The lowest curves represent the heart period response, where there is one pulse in each burst. As the period between stimulation decreases, the heart period increases. Note in both animals, there is a slight change in the configuration of the curve when the stimulation is about 500 msec. In the past, to achieve greater cardiac intervals, the frequency of the stimulation was increased.

We decided to increase vagal stimulation by varying the number of pulses in each burst, in addition to changing the base period. It is pertinent to remark again that this was achieved simply by typewriter entry. As the number of stimuli in each stimulation burst increased from 2 to 5, we noted that the mean heart period varied considerably from previous predictions. As the stimulation period decreases, the cardiac period in general increases. However, there are zones in which the cardiac period is the same period as the forcing function. Indeed, during these zones, the cardiac period decreases with the decreasing stimulation interval. As the number of stimulations in each burst increases, the length of the locking in-

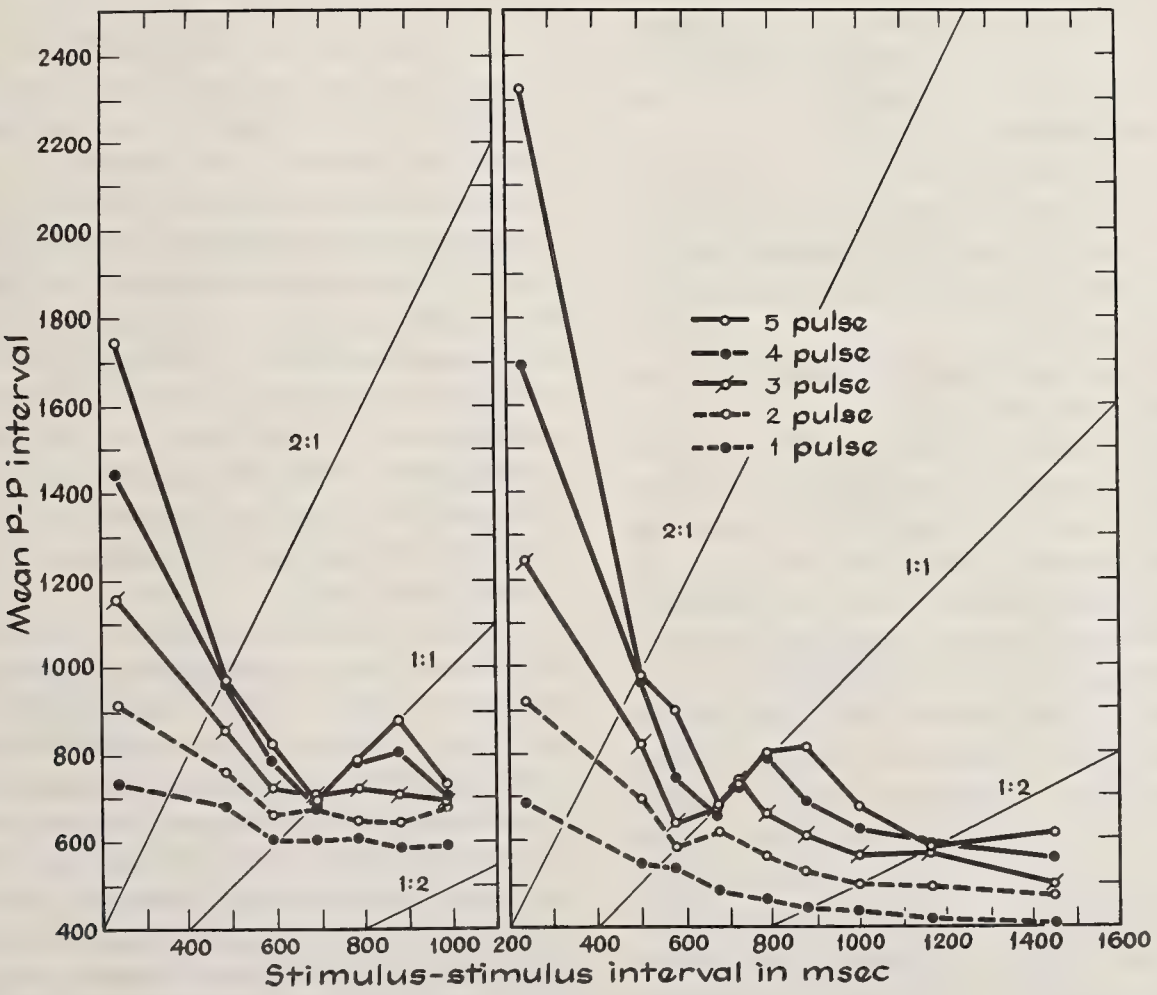


FIGURE 3.—Stimulation schematic.

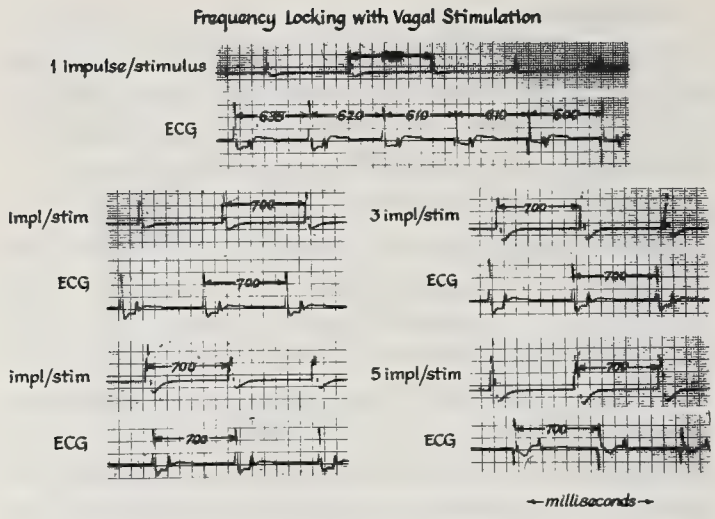


FIGURE 4.—Stimulus period versus mean heart period.

creases. Figure 3 also shows lines drawn to indicate the ratios 2:1, 1:1, and 1:2. Note that there is both harmonic and sub-harmonic coupling to the stimulation period.

Figure 4 shows a section of the records when the number of pulses in each burst increased but the period was held constant. Note that while there is greatly increased stimulation, the resulting heart period is unchanged.

These observations indicated to us that our conception of the vagal heart rate control subsystem was too simplistic. We then continued to examine in more detail this vagal-heart pacemaker sub-system.

The next experiment that we carried out was to stimulate the vagus, again using the digital computer as a control system to place the stimuli in specific phases of the cardiac cycle. The trigger in this instance was the "P" wave recorded from the atrial cavity. The computer detected the P onset (program lines 59 and 60). The 1800 then transmitted pulses to the laboratory and drove the Grass stimulator. The parameters, such as the number of pulses and the delay between the "P" and the stimulator, were all recorded. The subsequent cardiac periods were detected and printed out on-line in the laboratory (program lines 61 to 68), and the data were stored sequentially in the disc file (program line 70 to 73). An incremental plotter presented a hard copy of raw and mathematically transformed data on-line.

These features were particularly useful. The first advantage was to judge the quality of the data (program line 69), whether or not the computer had determined the P wave onsets correctly, in which case the data could be given permanently to the disc file. Second, the graphic presentation of the data permitted us to judge the confidence with which the data were being acquired. The human interaction with the graphic output permitted a reduction in the programming time, eliminating the development of sophisticated error detection schemes. The digital computer was useful because we could change the stimulation parameters through a wide variety of alternatives simply by typing in the appropriate values in the array and initiating the transfer to the 1800. It was possible to get a completely flexible system of experimental control and data reduction and storage with a minimum of program development. Approximately 1,000 stimulations were carried out in 18 dogs.[8] The time from the conception of the experiment to the first complete experiment was one work week. Actually, it took only four tries to develop the entire system and get it operational and on the air. It took us another three months, however, to collect all the experimental data.

The influence of the timing of vagal stimulation for heart rate is depicted in Figure 5. A burst placed about 200 milliseconds after the P wave onset results in a prolonged P–P interval. A burst placed later in the cycle results in no prolongation of that cycle, and a diminished prolongation of the subsequent beat.

Figure 6 summarizes the effect of varying the number of pulses and the timing of those pulses on the heart period. P represents time zero of the cycle in which the stimulus was carried out. The vertical axis represents the period in which the stimulation was carried out. Thus, in this example, a stimulus with one pulse placed at time zero, coincident with a P wave results in a cycle length of 550 msec. If the stimulus is placed 300 msec after the P wave, then the cycle is not inhibited. However, the following cycle is. We have elected to plot that effect as being placed before the reference P wave. As the stimulation is placed closer and closer to the reference P wave, the inhibition of that

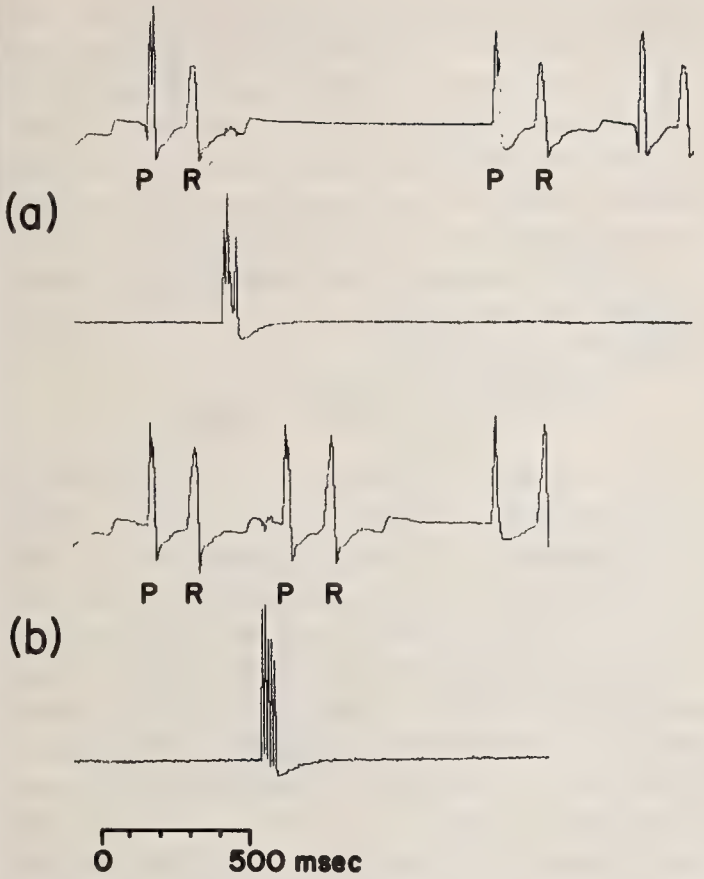


FIGURE 5.—EKG records of the effect of multiple stimuli.

cycle is increased. Increasing the number of stimuli in the stimulation changes the configuration of the total response curve as given in the second curve, where the number of stimuli in the stimulation is 3. There is still a time period near 200 msec before the end of the cycle in which this degree of inhibition results in no prolongation of the contained heart beat. These data show that the cardiac cycle is not temporally uniform in its response to vagal stimulation.

Now, the question arises: Is there a relationship between the first observation concerning frequency locking and the second observation of phase sensitivity?

If we examined the phase relationship between the vagal stimulation and the P wave onset in the first experiment we carried out, you will note that as the frequency of the vagal stimulation increases during the period of frequency locking, the time relationship between the stimulation and the P wave varies. The phase is shifting in a direction predicted by the phase sensitivity plots; as the stimulation frequency increased, the stimuli are placed in a phase of a cardiac cycle in which that same level of stimulation will result in less inhibition: thus, an increased heart rate rather than a decreased heart rate is achieved.

Now, what is the implication of these observations for the heart rate control system? In cardiac transplantation the donor heart containing the atrium and ventricle are excised and implanted in the new host, thus simulating an artificial heart. In our system of transplanting the heart, the atria are left behind. The donor ventricles beat independently of the recipient atria each having their own pacemaker. A typical intra-atrial recording demonstrating the electrograms in a human transplant is shown in Figure 7. The two components, donor and recipient, were separated out by computer methods. The two rates and the phase relationship where then plotted on the incremental plotter (Figure 8).

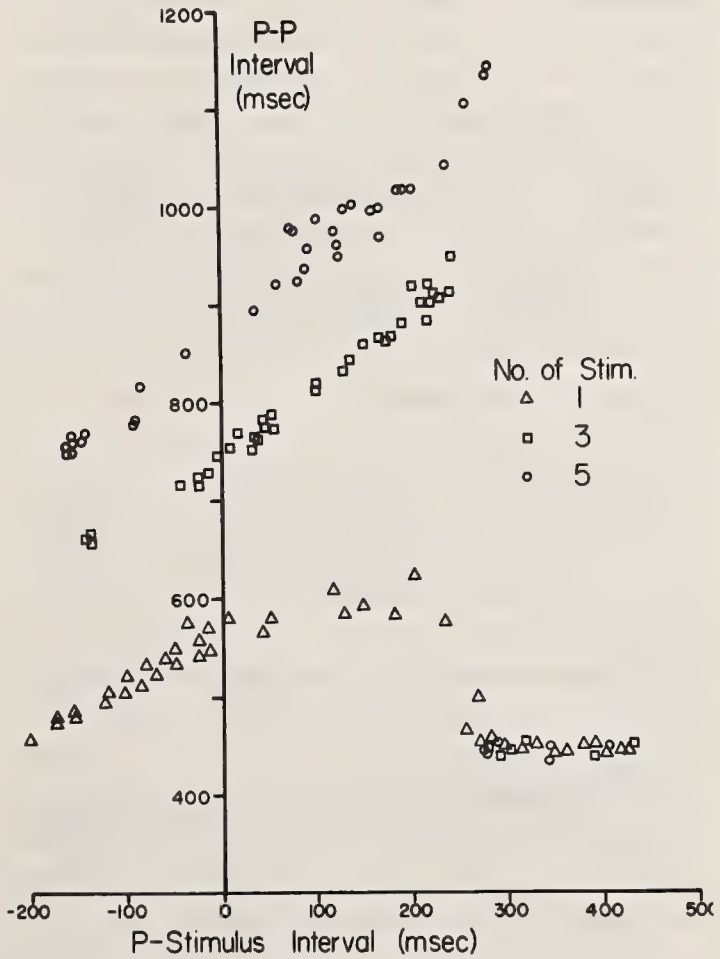


FIGURE 6.—Effect of timing on heart period.



FIGURE 7.—Intra-atrial EKG.

Figure 9 traces the course of the heart of a human heart transplant patient during exercise. The circles are the donor RR intervals and the XX's are the recipient P–P intervals. At the beginning, the P waves of the recipient and the R waves of the donor are independent in frequency and in phase. As the exercise increases and the donor heart rate increases, you will see that the two lock not only in frequency but in phase. The implication, therefore, is that the sino-atrial node of the human has the same type of behavior as the dog in the sense that it can lock to a driving arterial pressure transmitted through the central nervous system to the vagus. Besides its physiological implications, I feel that the data have direct application to the artificial heart devices. It may well be possible that the independent pulse generator in the system, under certain conditions of pressure and vagal transmission, forces the natural heart to beat in frequency with the artificial heart without any specific physical electrical linkage.

DECAY OF HEART RATE

We now wish to discuss the rate at which the heart period returns to the control state after cessation of vagal stimulation. In our initial discussion it was suggested that the decay of heart rate back to the control state could fit with a single exponential. Previous data suggested that the heart period was linearly proportional to ACH concentration. Therefore, during the carrying out of the previously mentioned experiments in phase sensitivity, the logarithm of the slope of the heart period was also displayed, utilizing two features of ACME system—on-line plotting and calculations (program lines 14.6 to 18). Figure 9 illustrates the typical heart response to stimulation and cessation of stimulation. Several trials were superimposed, illustrating the reproducibility of the experimental data, and our confidence in the adequacy of the data. We were immediately

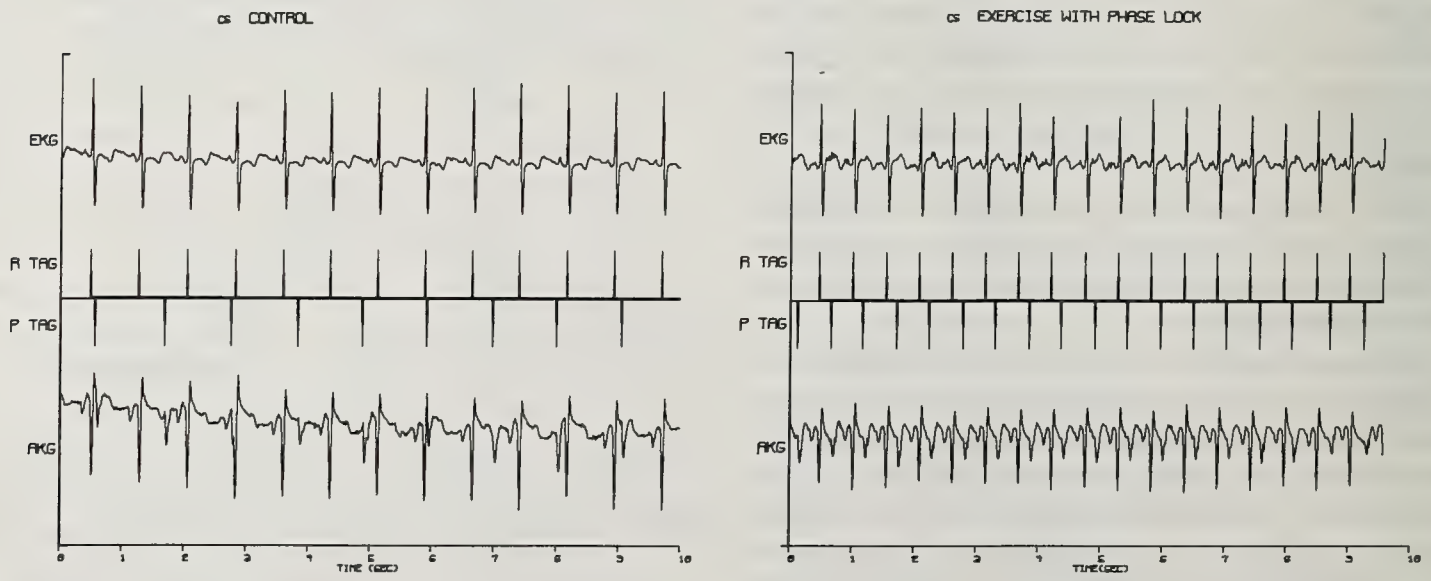


FIGURE 8.—Frequency relationships in transplanted hearts.

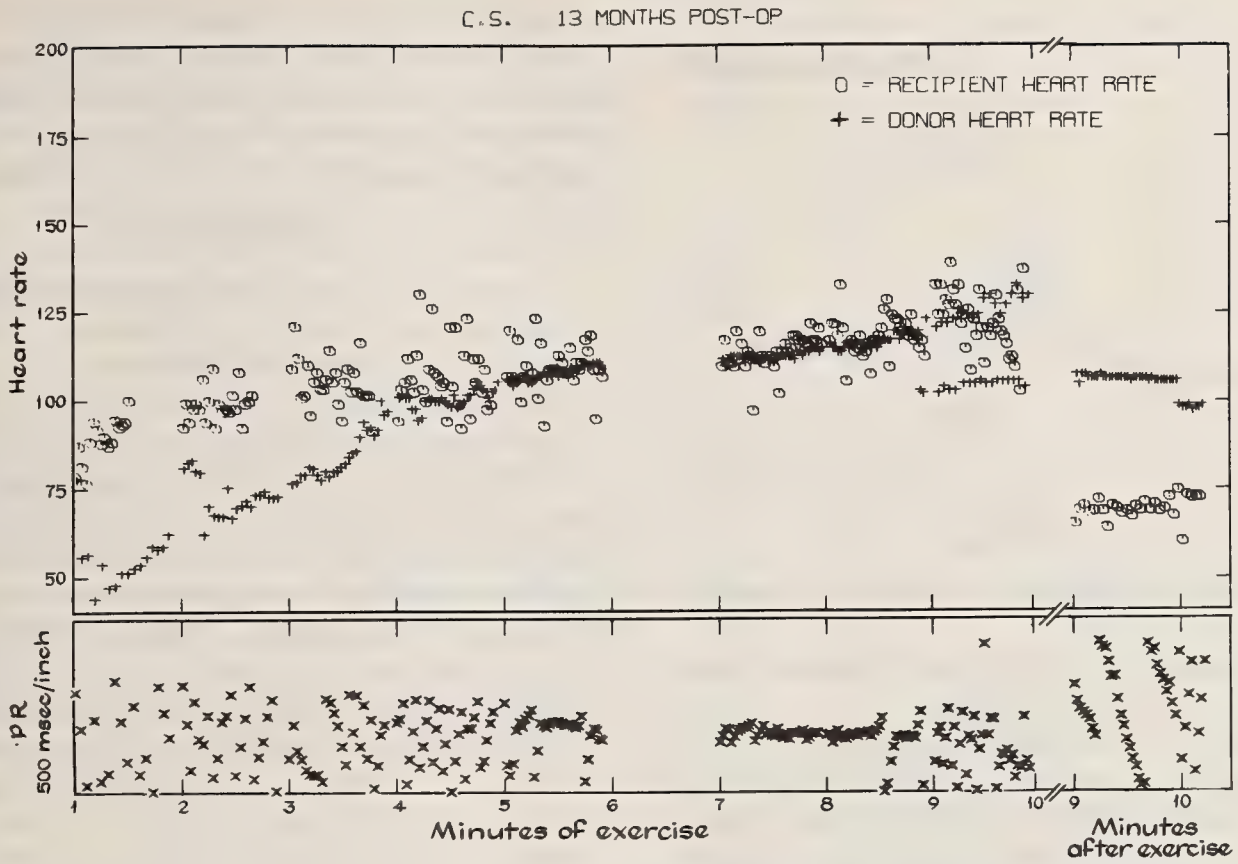


FIGURE 9.—Heart frequency response in exercise. Heart response to cessation of stimulation.

struck by the observation that the rate constants after the first two beats were relatively consistent, but the change of heart period immediately following the inhibited beat were consistently greater. These plots shown in figure 10 calculated the rate constant, plotted the experimental data, the best fit exponential and the residual.

A single exponential will increase the overall residual error, fitting neither the early nor late phases.

In pursuit of examining the heart rate control by the vagus, we have demonstrated the following features: (1) experimental control, (2) data storage and measurement, (3) graphic display, (4) curve fitting, and (5) use of statistical libraries. In order to formulate our understanding of the process involved we now turn to model building.

A well-known model of nerve potential in the axon has been proposed by Hodkin and Huxley.[9] This model relates alterations in cell membrane permeability to membrane potential. Noble, after experiments on Purkinje tissue, modified the axon model to fix the properties of the cardiac pacemaker—the spontaneous depolarization leading to rhythmicity.[10,11] We implemented this model on ACME.

Trautwein has demonstrated that acetylcholine causes a marked change in potassium permeability in cardiac tissue which is the basis of the inhibitory effect of ACH.[12] We therefore perturbed potassium permeability in the Noble model with a pulse decayed with a single exponential. You must remember that the Noble model was built to stimulate the membrane potential and not the beat-to-beat heart rate. Two problems arose. First, when given a time constant of 2.7 seconds, the model went into arrest with multiple inhibitions. Second, when the model was driven as if excited by an electrical simulus, the model returned to the control heart rate within one beat after discontinuation of the stimulus. These data were modeled by adding an RC circuit across the model.[13] This modification suggests that during pacing there is a net loss of positive charge. Following cessation

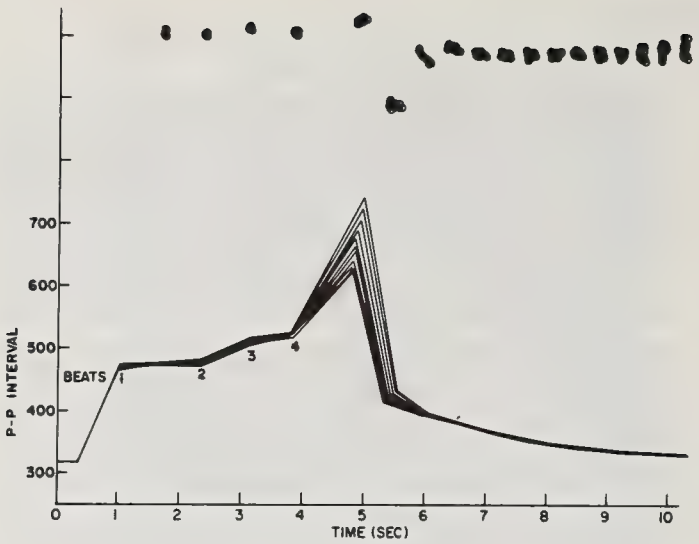


FIGURE 10.—Rate constants of return to normal size.

of the stimulation, while the imbalance exists, the heart period is prolonged.

Figure 11 represents a simulation for artificial pacing. The top figure is the voltage across the membrane. In the lower left is the voltage across the RC circuit as stimulation progresses. On the right half of the figure are values for E_M and E_{C1} following cessation of the pacemaking, illustrating the fall in E_{C1}, the depressed slope of the prepotential, the so-called post-pacemaker depression—a long beat and the slow return to control heart period.

Further simulated acetycholine experiments were carried out to find the parameters of the equation, using the interactive graphic features. Each simulation ran about 15 minutes with output on the oscilloscope. Observing the waveform permitted educated guessing and shortened the time for solution. Extensive simulation experiments were then carried out perturbing both the potassium and sodium conductances with an exponential function with varying time constants.

Figure 12 illustrates the results of one such simulation. Again the top row gives the simulated membrane potential, the second row the change in potassium current simulating the acetylcholine pulse, and the bottom is the postulated voltage across the E_{C1}. The features are a prompt inhibition, a depressed prepotential, and a gradual return to control period. Figure 13 illustrates the logarithm of the period following the simulated vagal inhibition, which resembles the data achieved by the very rapid initial change in the heart period in this case, a slope of .6 sec followed by an exponential of significantly greater value (2.6 seconds). The model also demonstrates phasic responsiveness.

SUMMARY

A case study has been presented in the use of a general-purpose, time-shared computer system for physiological research. The experiments used computer-controlled stimulations to demonstrate that the heart will lock in phase and frequency to a forcing vagal stimulation frequency. Graphics and controlled stimulation demonstrated that the cardiac cycle is temporarily non-uniform in its response to brief vagal stimulation. Long-term storage of data, curve fitting, and use of statistical libraries accelerated the demonstration that there are two phases as the inhibited heart rate returns to a control state. The later phase does fit an ex-

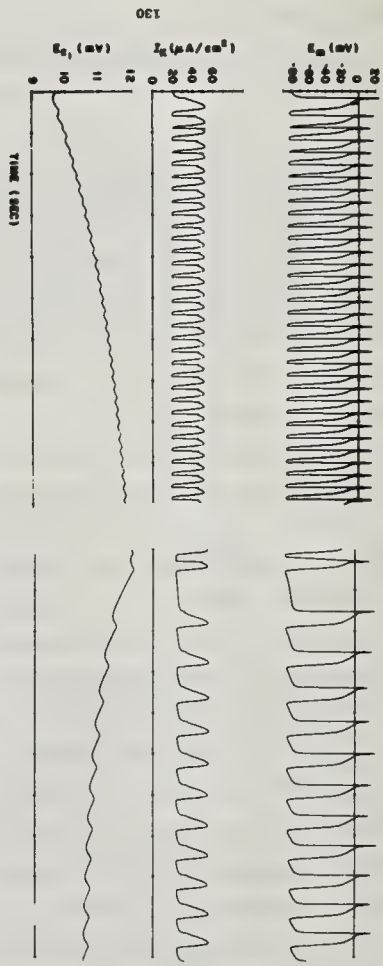


FIGURE 11.—Simulation for artificial pacing.

ponential function with a time constant from 2.6 to 4.8 secs.

Lastly, experiments in computer simulation suggested a change in the current model of pacemaker function which, when modified, simulated not only the currently acquired biological data but also simulated such known phenomena as post-pacemaker depression.

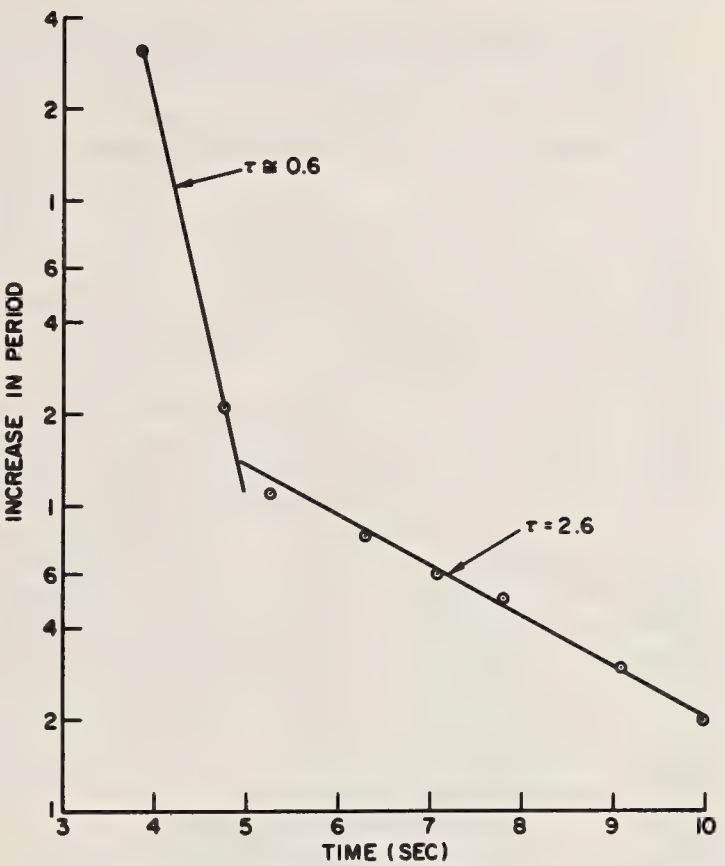


FIGURE 13.—Heart period response to vagal stimulation.

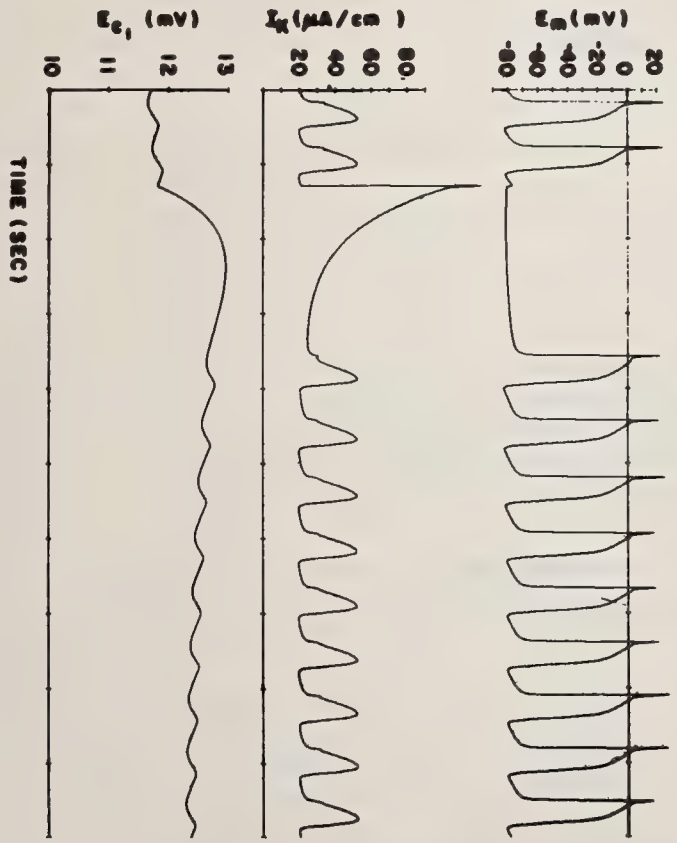


FIGURE 12.—Simulation for acetylene pulse.

REFERENCES

1. SANDERS, W. J., et al. An Advanced Computer System for Medical Research. AFIPS Conference Proceedings Vol. 31, Anaheim, Calif., 1967.
2. WIEDERHOLD, G. An Advanced Computer System for medical Research. *Proceedings of the IBM Japan Computer Science Symposium-Research and Computer System,* Tokyo, Japan pp. B1–B15, November 1969.
3. BREITBARD, G. Y. and WIEDERHOLD, G. PL/ACME: An Incremental Compiler for a Subset of PL/1. *IFIP68 Congress Proceedings* Edinburg, Scotland, August, 1968.
4. GIRARDI, S., FREY, R. and WIEDERHOLD, G. A Filing System for Medical Research, presented at Journees Internationales d'informatique Medicale de Toulouse, France, March 4–6, 1970, and at The Eight Annual Symposium on Biomathematics and Computer Science in the Life Sciences, Houston Texas, March 23–24, 1970.
5. WIEDERHOLD, G. and HUNDLEY, L. A Time-Shared Data Acquisition System Digest 1969 IEEE Computer Group Conference on Real-Time Systems, IEEE, Minneapolis, Minn., pp. 190–196, June 17, 1969.
6. CROUSE, L. and WIEDERHOLD, G. An Advanced Computer System for Real Time Medical Applications, *Computers in Biomedical Research* (Academic Press), Vol. 2, Number 6, Dec., 1969.
7. WARNER, H. R. and RUSSELL, R. O. Effect of Combined Sympathetic and Vagal Stimulation on Heart Rate in the Dog. Circ., 24, 1969.
8. DONG, E. and REITZ, B. The Effect of Timing of Vagal Stimulation on Heart Rate in the dog. Circ. Research 27:635, 1970.
9. HODGKIN, A. L. and HUXLEY, A. F. Quantative description of membrane current and its application to conduction and exitation in nerve. J. Physiology RL 403–414, 1952.
10. NOBLE, D. A Modification of the Hodgkin-Huxley Equations Applicable to Purkinje Fibre Action and Pacemaker Potentials. J. Physiol., 160, 317–352, 1962.
11. MCALLISTER, R. E. and NOBLE, D. The Time and

Voltage Dependence of the Slow Outward Current in Cardiac Purkinje Fibres. J. Physiol., 186:632–662, 1966.
12. Trautwein, W. and Dudel, J. Aktionspotential und Mechanogramm des Warmbluterherzmuskels als Funktion der Schlagfrequenz. Arch ges. Physiol. 260, 24:39, 1954.
13. Weaver, C. S. and Dong, E. A phase sensitive model for vagel heart rate control. J. Theoretical Biology (in press)

Appendix: A Vagal Stimulation Program

```
 1.000 VAG—STIM: PROCEDURE;
10.000 declare (a,b,pulse,pulsel) (250); onevolt=6000;
11.000 declare storebuf(50,21),outbuf(25);
11.500 declare name char(8);
12.000 declare EKGin initial(61), p—in initial(62), p—out initial(97);
12.500 /* set up proper buffersize(250),startcode(1=read when opened) */
13.000 call SETDIR(EKGin,8,5,250); call SETDIR(EKGin,8,6,1);
13.500 /*          and sampletimeinterval(2 milliseconds),buffernumber(60) */
14.000 call SETDIR(EKGin,8,7,2); call SETDIR(EKGin,8,8,60);
14.500 call SETDIR(p—in,8,5,250); call SETDIR(p—in,8,6,1);
14.600 call SETDIR(p—in,8,7,2); call SETDIR(p—in,8,8,60);
14.700 call SETDIR(p—out,8,5,250); call SETDIR(p—out,8,6,1);
14.800 call SETDIR(p—out,8,772); call SETDIR(p—out,8,11,260);
14.900 declare plot—out initial (13); call plotopen(plot—out);
15.000 declare LT(10) char(1); do i = 1 to 9; LT(i) = CHAR(i); end;LT(10)='0';
15.100 pen0=0;penfin=1;pendraw=2;penmove=3;
15.200 limit:procedure(val,max,min);
15.300     if val>max then return(max);
15.400     if val< min then return(min);
15.500     return(val); end limit;
15.600 /* all dimensions in terms of hundreths of inches */
15.700  paper=900;border=20;let—size=20;xoff=100;newsheet=2550;
15.800 drawframe:procedure;
15.900   call plot(plotout,0,0,pen0);
16.000     if havesetpen then call plot(plotout,newsheet,0,penmove);
16.100         else do; pause; havesetpen = 1; end;
16.200     T = 1; call dispt(700, 'TIME(MSEC)');
16.300     call dispy(2,100,8,1,300,'PP–INT(MSEC)');
16.400     call plot(plotout,paper,0,penmove);
16.500     T = .01; call dispt(1400,'TIME(SEC)');
16.600     call  dispy(1,200,4,1,300,'PP–INT(MSEC)');
16.700     call dispy(2,2,4,6,–4,'SLOPE');
16.800     call plot(plotout,1000,800,pendraw);
16.900     call plot(plotout,–paper,0,pendraw);
17.000     call plot(plotout,0,0,penfin); call plot(plotout,0,0,pen0);
17.100     end drawframe;
17.200 rat1 = 100; min1 = 300;
17.300 rat2 = 200; min2 = 300;
17.400 drawnew:procedure;
17.500     x = outbuf(2)*100;
17.600     y = 100 *(outbuf(9)–min1)/rat1+border;
17.700         y = limit(y,1000,xoff);
17.800     call  plotsymbol(plotout,x,y,let—size,LT(outbuf(1)),0,0);
17.900     call plot(plotout,0,0,penmove);
17.910     call plot(plotout,papersize,0,penmove);
17.920     call plot(plotout,0,0,pen0);
17.930     x = 100;
17.940     y = 100 *(outbuf(3)–min2)/rat2+xoffs;
17.950         y = limit(y,600,xoff);
17.960     call plot(plotout,x,y,penmove);
17.970     do l = 3 to 20;
17.980         x = x + outbuf(l)/10;
```

```
17.990          y = 100 *(outbuf(1)-min2)/rat2+xoffs;
17.991              y = limit(y,600,xoff);
17.993      call plot(plotout,x,y,pendraw); end;
17.995      call plot(plotout,0,0,penfin);
18.000  end drawnew;
18.500
19.000  test:procedure; /* test stimuli, called from terminal */
19.500      pulse = 0; pulsel = 0; /* 500 pulses at 2 millisecs each */
20.000      pulse(1),pulse(4) = onevolt;
21.000          do k = 1 to 1000;
22.000              call write(p—out,pulse); call write(p—out,pulsel);
23.000              end;
24.000              end test;
25.000  hit:procedure; /* set up and send two buffers (1 sec.) of stimuli */
26.000      do s = 1 to ns;
27.000          if s=1 then do; if (q+step)>250 then cflag = 250; else cflag = 0; end;
28.000          c = q+step+(5*(s-1))-cflag;
29.000              if c>250 then pulsel(c-250) = onevolt; else pulse(c) = onevolt;
30.000              end;
31.000              call write(p—out,pulse); call write(p—out, pulsel);
32.000              ncnt = 50;
33.000      end hit;
34.000  initial:procedure;
35.000      call close(EKGin); call close(P —in);
36.000      a,b,pulse,pulsel,rcnt,counter,rtime,bflag = 0;
37.000      i,lastcount = 1;
38.000      ncnt = 3;
39.000      end initial;
39.100    dispy:procedure(INCR,RATR,DIV,OFFS,VMINR,TITLEY);
39.110      declare TITLEY char, SC char(4);
39.120      xoffs = 100*OFFS;
39.130      call   plotsymbol(plotout,border,xoffs+border,let—size, TILLEY,90,0);
39.140      do j = 0 to DIV by INCR; SC = CHAR(VMINR+j*RATR);
39.150          call   plotsymbol(plotout,border+10,xoffs-10+j*100,let—size,SC,0,0);
39.160          end;
39.170      call plot(plotout,0,0,penfin); end dispy;
39.200  dispt:procedure(M,TITLET);
39.210      declare TITLET char,TIME char(4);
39.220      do j = 1 to 20;
39.230          if M>j*100 & M<=(j+1)*100 then do;
39.240              NODR = j+1; go to LEP; end;
39.250      end;
39.260      LEP:do j = 0 to NODR; jjj = 100*j+1;
39.270          call    plot(plotout,jjj,border+30,penmove);
39.280          call plot(plotout,jjj,border+55,pendraw);
39.290          TIME = char(trunc(j*T*100+.005));
39.300          call  plotsymbol(plotout,jjj—25,25,let—size,TIME,0,0);
39.310      end;
39.320      call   plotsymbol(plotout,300,0,let—size,TITLET,0,0);
39.330      call plot(plotout,100,0,penfin);
39.340      end dispt;
39.500
40.000  threshold,step,z = 0; blank = 250;
41.000  get data('number of steps to skip', nstep);
42.000  get data('number of stimuli to increment', nsstep);
43.000  get data('starting number of stimuli',ns);
44.000  get data('filename (8 characters)',name);
45.000  get data('number of tests before incrementing stimulus',nburst);
46.000  begin: call initial;
46.500  call drawframe;
```

```
47.000 read1: call open(EKGin); call open(p—in);
48.000 read2: call read(EKGin,a); call read(p—in,b);
49.000 counter = counter + 1;
50.000 if counter = 1 then go to read2;
50.900 /* look for a stimulus output read back in */
51.000 do q = 1 to 250;
52.000      rtime = rtime + 2;
53.000      if bflag=1 & b(q)>threshold then bflag = 0;
54.000 btest: if b(q)<threshold & bflag=0 then do;
55.000           if i>1 then do; bflag = 1; nstim = nstim + 1; go to rtest; end;
56.000           outbuf(2) = rtime; ncnt = 50; i = 50; i = 2; nstim = 1; bflag = 1;
57.000 end;
58.000 /* now look for the R-wave */
59.000 rtest: if a(q)<threshold & rtime>blank then do;
60.000      rcnt = rcnt + 1; if rcnt>ncnt then call hit;
61.000      if i>1 then do; outbuf(1+2) = rtime; i = i + 1; end;
62.000      else outbuf(3) = rtime;
62.500      rtime = 0;
63.000      end;
64.000 output:if i>22 then do;
65.000      outbuf(1) = nstim;
66.000      call initial;
67.000      put image((outbuf(j) do j = 1 to 20))(im1);
68.000      im1:'
68.500 call drawnew;
69.000      get data(okay);
70.000      if okay then do; open file(fn) title(name) output;
71.000                    write file(fn) from(outbuf);
72.000                    close file(fn);
73.000                  end;
74.000      step = step + nstep; z = z + 1;
75.000      if z>nburst then do;
76.000          ns = ns +nsstep; z = 0; step = 0; pause; end;
77.000      go to read1;
78.000      end;
79.000 end;
80.000 if counter>60 then go to read2;
81.000 call close(EKGin); call close(p—in); put list(counter);
82.000 counter = 0;
83.000 call open(EKGin); call open(p—in); go to begin;
84.000 end VAG—STIM;
```

DISCUSSION

P. G. KATONA, Case Western Reserve University, Cleveland: I wonder if you'd elaborate on your statement that this might be useful for driving the natural heart from an artificial heart? I was wondering why you found it useful to do it that way rather than driving the artificial heart from the natural heart.

DR. DONG: I didn't make any comment on the usefulness of it. I'm just commenting on the fact that it is possible for that to occur. If you're taking an assist device and not synchronizing it to the natural heart and you think you have two pulse generators working independently, under certain conditions, it is possible to force the natural heart to beat to the driving, artificial heart without any electrical or physical connection. It's possible that you'll drive it through the baroreceptor and through the central nervous system. I remember, and I haven't been able to identify it (perhaps somebody in the audience can), an observation made by the Houston group in which they did such a thing. They permitted the artificial heart to beat independently of the natural heart, and they made an observation that at around 60 beats a minute the two hearts (one artificial and one normal) would synchronize.

D. B. JACKSON, Abbott Laboratories, North Chicago: You mentioned in your abstract that you're now using a small computer. Is this system you describe small? I mean this wasn't a small system. . . .

DR. DONG: No.

DR. JACKSON: I wondered what it was?

DR. DONG: We carried out all these experiments that I described here on ACME which is a 360–50 with an 1800. Since that time, we have purchased a PDP-12, and we started to do the same type of experimentation. As I said, it took us four days to carry out the first experiments with the ACME system. It took us six weeks to program the machine line in which to carry out the same thing on a PDP-12. For reasons of economy, it was cheaper to do it on the PDP-12 when you're going into a production mode.

CHAIRMAN WARNER: I'd like to just make a couple of comments about your observations of phase sensitivity of the vagal stimulus on heart rate and its relation particularly to the model you presented initially. Bob Tuckett in our laboratory has been studying this phenomenon for a couple of years and has done many of the same kinds of things you describe here. But, he has gone a step further I suppose in the sense that he's tried to take this observation and test it against the model. He found that by altering the model so that heart rate now, instead of being a function of frequency of stimulation, changes the equation to be a discrete function, where each stimulus represents a unit input to the model. With that addition, there is only one other parameter, which is a parameter that describes the delay in transmission of the signal from the point of stimulation on the vagus to the sinoauricular node. This model does describe the phase sensitivity.

I differ with you in one comment you made, and that was that the important part of the model is the proportionality between acetylcholine concentration and heart rate. Of course, that is an element in the model. But the interesting features of the model are the kinetics, the dynamic features of the relationship of the rate of destruction for acetylcholine and the release of acetylcholine at the time the stimulus arises. These two phenomena will account for the features that you present in your experimental data. It might be interesting to test your data against that same phenomena. Would you like to make any closing remarks?

DR. DONG: Time didn't permit the demonstration of a different model which we modified. There is a well-known axone model of Hodgkins and Huxley which was modified by Noble to simulate the membrane potential. We modified that model too by adding a simple circuit to it, and simulated the whole thing on the ACME system. It also will describe phase sensitivity. Although the decay of heart rate from stimulation to control state was previously described, I think, as a single exponential (and experimental data does not confirm that), it appears as if the decay of heart rate from stimulated state to control state is actually at least two phases: one having a very slow phase and one having a very, very fast phase. This modification was added to Noble's model so that it can simulate that, as well as phase sensitivity. What I want to say here today was simply that the use of this large system has given us great insight and control over acquiring data as well as analyzing data and I think that it's extremely useful for physiological research. It is well to have both of these types of systems as well as other more dedicated systems.

CHAIRMAN: Thank you Dr. Dong. I will just conclude by saying there is no such thing as a unique model to any system and the final criteria for selection of a model for describing a particular phenomenon must be based on aesthetics.

BIOTELEMETRY TRANSMITTER DESIGN METHODS

V. G. Ellerbruch,* F. C. Fitchen* and R. W. Sawrey**

When telemetry transmitters are designed for operation in the UHF frequency range, classical lumped-parameter theory cannot be easily applied. Simple passive circuit components, like resistors, appear as complex impedances, and active devices, such as transistors, exhibit frequency sensitive gains and phase angles. These deviations from the ideal complicate transmitter design.

Crystals, at these frequencies, cause a special problem because of their narrow bandwidths and the ever present capacity. Also, crystals cannot be cut to place their fundamental frequency in the UHF range; those that are used operate on overtones. Since many overtones exist that are quite close together, the circuit must be designed to select the correct frequency. It is true that the drive level should not be an important factor when designing with crystals, as long as a maximum power level is not exceeded; however, for practical low energy circuits such as those being used in biotelemetry systems the drive level must be considered. Data is given showing how crystal impedance depends on drive level.

This paper describes design methods that do not require complete lumped-parameter component models. Transmission line techniques are used with the elements in the design represented by reflection coefficients or s-parameters. The measured data are plotted on impedance diagrams which are used as aids in the design process.

A design example is used to illustrate the methods and data are given to show the effects of encapsulating material. In general, encapsulating materials cause frequency shifts which are shown to be functions of curing time and coating thickness.

For FM transmitter design, one can obtain the desired frequency deviation by using purely resistive transducers. By using care, the circuit does not have to be overly complicated to realize such a design.

INTRODUCTION

Designing telemetry transmitters can be a frustrating experience, even for the seasoned designer. There are several reasons why transmitter design presents special challenges.

The feeling of being overwhelmed sometimes sets in when a designer realizes the stringent requirements of his system; limited physical size, hostile environments, minimum expendable energy, long operational life and economic feasibility. This last condition is not the least to be considered because it forces the use of standard tolerance, off-the-shelf components.

It is usually impossible to purchase stock electrical components where the manufacturer gives all the pertinent data. Perhaps the manufacturer can supply such information but the cost of doing so is passed along to the user, and may be prohibitive.

This paper describes a design method, that relies upon use of a set of easily measured s-parameters. The method does not require that one have available a complete analytical description for each component as a function of frequency or biasing, but by making careful measurements and employing graphical techniques a satisfactory design is achievable. The method allows for close examination of limiting factors such as battery drain, environmental effects, component tolerances and the like.

BASIC THEORY

The development of very high frequency semiconductor devices has stimulated considerable use of the scattering parameters. These parameters are commonly known as s-parameters. The use of s-parameters has generally been confined to high frequency (greater than 100 MHz) work; however, such a limitation is not essential as s-parameters have been used for circuit design at frequencies as low as 10 MHz. The use of s-parameters for 2-port networks has some distinct advantages over other parameters.

An important reason why s-parameters are

* Electrical Engineering Department, South Dakota State University, Brookings, South Dakota.
** FMC Corporation, Minneapolis, Minnesota.

becoming widely accepted is that they are easy to measure, because the required terminations are not open or short circuits as is true of the z- or y-parameters. The use of a finite terminating impedance for measurement helps to stabilize the device whose parameters are being measured; whereas the device may become unstable when an attempt to short or open one port is made. This advantage is especially evident at frequencies above 100 MHz where valid open or short circuits are difficult to obtain.

In system design the transfer of power is generally of primary interest and mating networks are often used so that power transfer is optimized. The s-parameters provide information that is closely related to power transfer; therefore, circuit design is simplified, because the required matching networks are easy to describe.

Numerous papers have defined and discussed the generalized scattering parameters and, therefore, a detailed description is not given here.[1-4] Material sufficient for understanding how the parameters are used for the present application is included.

Figure 1 shows a 2-port network to be described by s-parameters. The equations that are generally used are

$$b_1 = s_{11}a_1 + s_{12}a_2 \qquad (1)$$
$$b_2 = s_{21}a_1 + s_{22}a_2$$

where the a's and b's are the incident and reflected voltage waves normalized to $\sqrt{Z_o}$. The characteristic impedance is designed by Z_o. The incident and reflected waves are closely related to power flow, since $|a_i|^2$ is the power incident on the i^{th} port and $|b_i|^2$ is the power reflected from or leaving the i^{th} port.

If a_2 is set to 0, s_{11} and s_{21} may be determined; and if a_1 is 0, then s_{12} and s_{22} may be determined. Either a_1 or a_2 is made 0 by terminating the port of interest in Z_o. This termination absorbs all the incident power, so there is no reflected wave.

If V^+ and V^- are defined as the incident and reflected voltage waves, respectively, then the following definitions hold and can be used to find the s-parameters.

$$s_{11} = V_1^-/V_1^+\Big|_{V_2^+ = 0} \qquad s_{12} = V_1^-/V_2^+\Big|_{V_1^+ = 0}$$
$$s_{21} = V_2^-/V_1^+\Big|_{V_2^+ = 0} \qquad s_{22} = V_2^-/V_2^+\Big|_{V_1^+ = 0} \qquad (2)$$

The incident and reflected voltages are easily measured. The parameters s_{11} and s_{22} are simply reflection coefficients and are measured under the condition that both ports are terminated in the characteristic impedance of the system, Z_o They provide sufficient information for determining the input and output impedances of a 2-port system, when Z_o terminations are used. Likewise, the parameters s_{21} and s_{12} are the forward and reverse transmission gains with the input and output ports terminated in Z_o.

Eq. (1) shows that like other parameter sets, s-parameters relate two input and two output quantities, and Eq. (2) indicates the conditions that must be satisfied to measure the four parameters. Figure 2 shows that the terminals of the 2-port device connected to a system that has a characteristic impedance of 50 ohms. The 50-ohm terminations insure that no reflections will occur at the load or generator. By applying a signal to one of the ports, 3 measurements will define 2 of the parameters. If, for example, the input is on the left in Figure 2, then $V_2^+ = a_2 = 0$. Under this condition, measurements of V_1^+, V_1^- and V_2^- will specify s_{11} and s_{21} as indicated by Eq. (2). If the input and output ports are reversed, then $V_1^+ = a_1 = 0$ and meas-

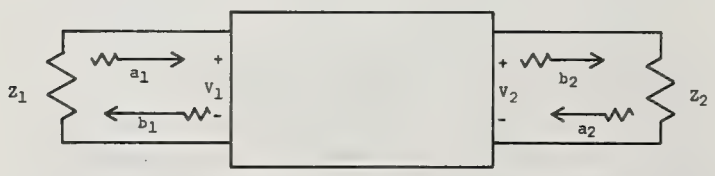


FIGURE 1.—Two-port network. Biotelemetry transmitter design methods.

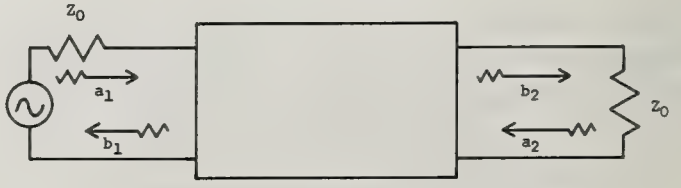


FIGURE 2.—Simplified measurement circuit.

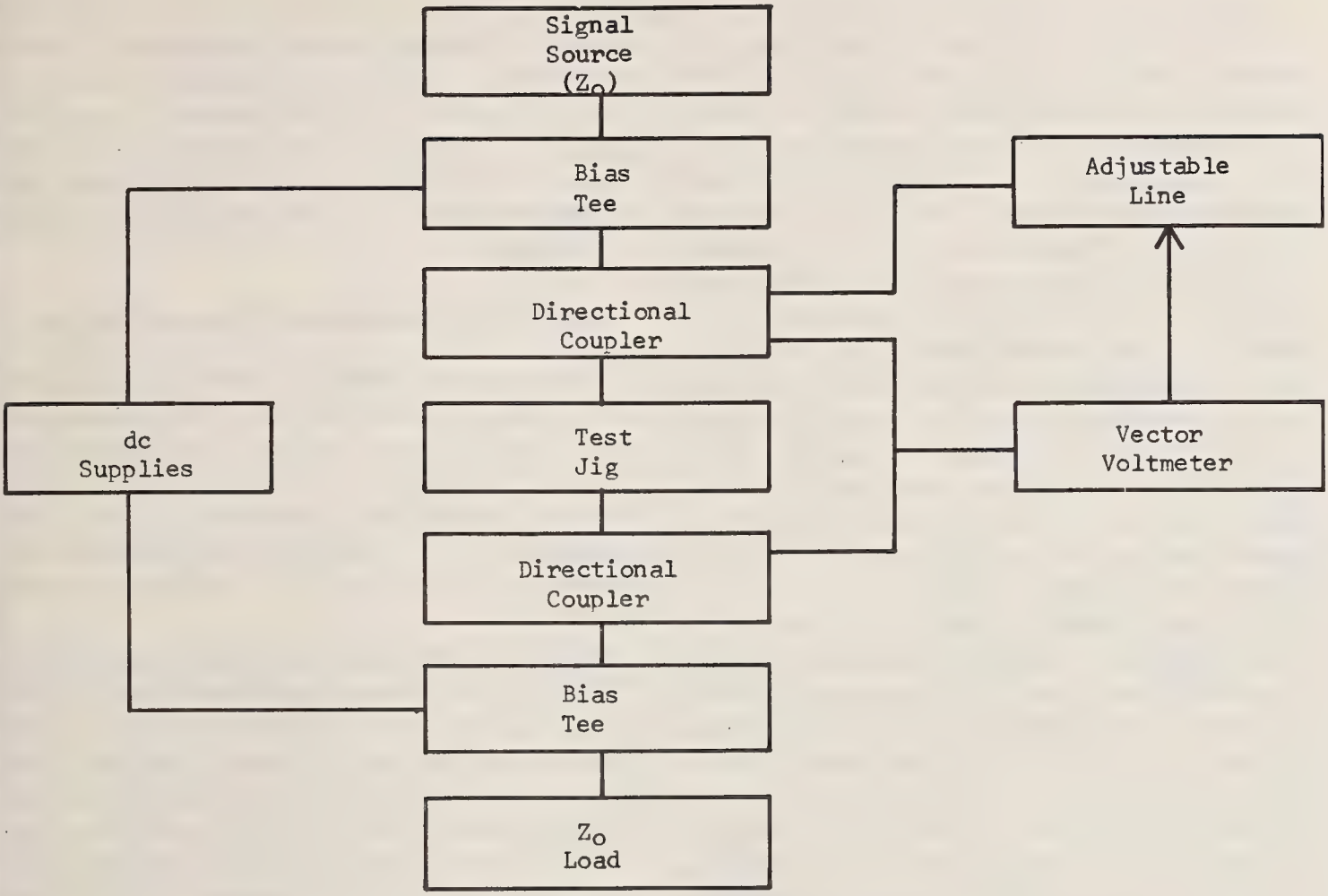


FIGURE 3.—Block diagram of measurement system.

urements of V_2^+, V_2^- and V_1^- will specify s_{12} and s_{22}. These six voltages completely specify the set of s-parameters for a given 2-port network. A more complete block diagram of the measurement system is shown in Figure 3.

DESIGN CONCEPTS

A simple biotelemetry transmitter can be visualized as consisting of 3 main parts; (1) active network, (2) input termination and (3) output termination. Figure 4 illustrates, in block diagram form, such a transmitter. The active network might consist of only one transistor plus biasing circuitry or it might consist of a complete subsystem such as a transistor combined with a filter network.

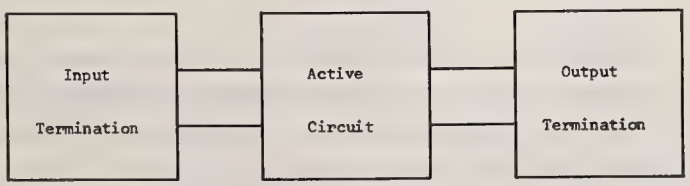


FIGURE 4.—Transmitter block diagram Biotelemetry transmitter design methods.

If more complex circuits are required, then the active network might be a complex integrated circuit (IC). The reason more ICs are not presently being used in the biotelemetry business is because they are expensive unless mass produced, and their power drain is often excessive. The power drain of an ordinary IC is usually prohibitive for applications requiring wireless implantable transmitters or for longevity transmitters such as those used on wildlife subjects that are allowed complete freedom of movement in their habitat.

The time should come when standard, low energy circuits will be readily available for biotelemetry systems. Then ICs will be economical from both energy and cost standpoints.

Typical active network terminations for transmitters might include the following: an antenna, amplifier, a biological specimen such as fluid or body tissue. Each termination, whatever it might be, has its own peculiar electrical properties that must be known before it can successfully be coupled to the active network. Knowing the properties of the port terminations in terms of the s-parameters gives the designer the specific information he must have for matching these terminations to the active network for achieving maximum power transfer. In many practical designs the terminations are known and cannot be modified so that the active network must be designed accordingly. A fixed input or output termination doesn't present an unusual design problem if there is, in the end, a physically realizable solution.

Of all the variables one can list for consideration of input and output terminations a few are of extreme importance. The frequency response and termination impedances are among those features that are often not considered in sufficient detail during the design of a biotransmitter. It is difficult, if not impossible, to obtain the data required for making an accurate analysis of the electrical characteristics of biological systems. Sometimes, the task is further complicated by the time varying nature of the biological system. In spite of all the drawbacks and indeterminants, measurements must be made and used to integrate the input and output terminations together with the active network into a compatible, reliable, and useful design. As much witchcraft as possible must be eliminated.

Input data can and do come from a variety of sources and each has its own individual characteristics that must be considered. ECG, EEG, myograms, pressures and temperatures are physiological functions that are monitored in great detail by a variety of techniques and electronic equipment. There are also any number of important measurements being made that are of keen interest to only a few individuals; specialized equipment is fabricated and standard procedures and equipment are not developed and available.

Electronic systems are designed to meet specific objectives and one must never lose sight of his objective during the design process. For example, a system may be designed as a signal conditioner where some filtering or amplification is required. In other instances it might be that data acquisition and transmission is the goal. It is the latter case that this paper discusses, and specific illustrative design example is included.

INDIVIDUAL COMPONENT MEASUREMENTS

It is easy to purchase resistors that do not have a reactive component at UHF frequencies, but when they are installed in a circuit, care must be taken to keep their lead lengths short. Figure 5 shows the impedance for a 50 Ω resistor that has 2.5 cm leads. For the same component with short leads the resistance appears as 50 Ω.

Inductors and capacitors, like resistors, have values that are functions of lead lengths. The inductors used in the designs discussed in this paper are of the molded ferrite core type; they are not hand-wound. Hand-winding is generally not satisfactory because of the variability from one coil to the next. The molded inductors are manufactured within a tolerance of 30% which is acceptable.

When several identical circuits are being fabricated it is best to use a printed circuit board if at all possible. Then, even if the components do not exhibit "pure values" because of layout, at least the contributions due to stray reactances will be repeatable.

One can spend much time studying the different variations in the s-parameters for transistors. Kim [5] has done such a study. Some data is given here to help the reader visualize transistor reflection coefficients as functions of frequency.

The 4 s-parameters for a UHF transistor are plotted in Figure 6; the A curves. The operating point for these data was $V_{CE} = .7$ V and $I_C = .1$ mA.

Data were taken (Figure 6, B curves) for the same frequency range, only the collector bias current was changed to $I_C = .5$ mA. The s_{21} parameter has undergone the greatest change because it is now greater than 1 and is not plotted.

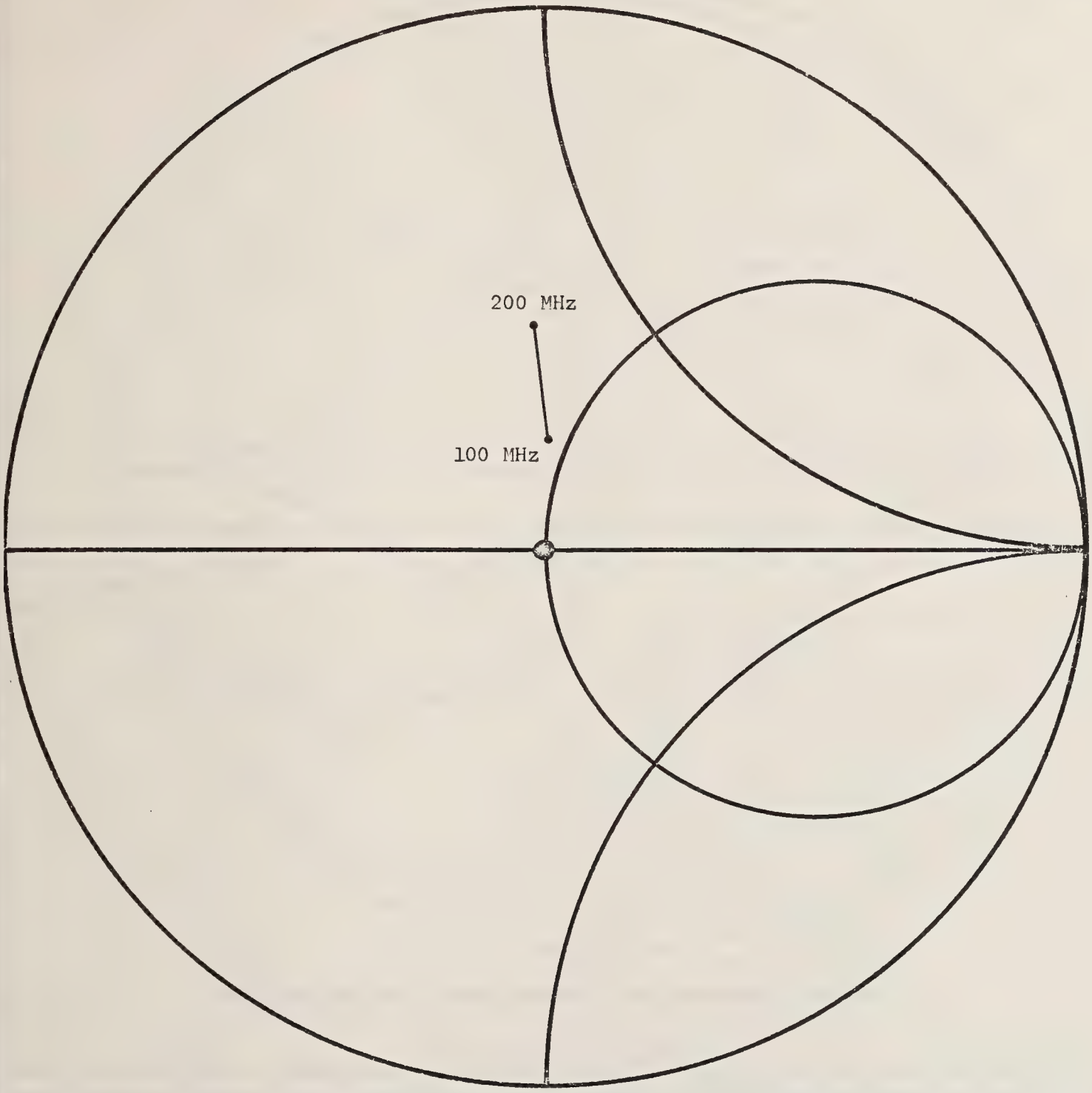


FIGURE 5.—Plot of a 50-ohm resistor.

Crystals cause severe problems for the designer because of the unit-to-unit variations in resonant frequency, series resonant resistance and shunt capacity. Even though the manufacturer gives accurate values for these 3 variables at the resonant frequency, the complete story is not told.

Figure 7 shows a plot of data on 2 crystals that were purchased from different manufacturers. Curve A is a crystal that was specified to have a series resonant frequency of 151.125 MHz, with a resonant impedance less than 100 ohms and a shunt capacity less than 7 pF.

Curve B shows the data for a crystal that was specified to have a series resonant frequency of 151.023721 MHz, series resonant resistance

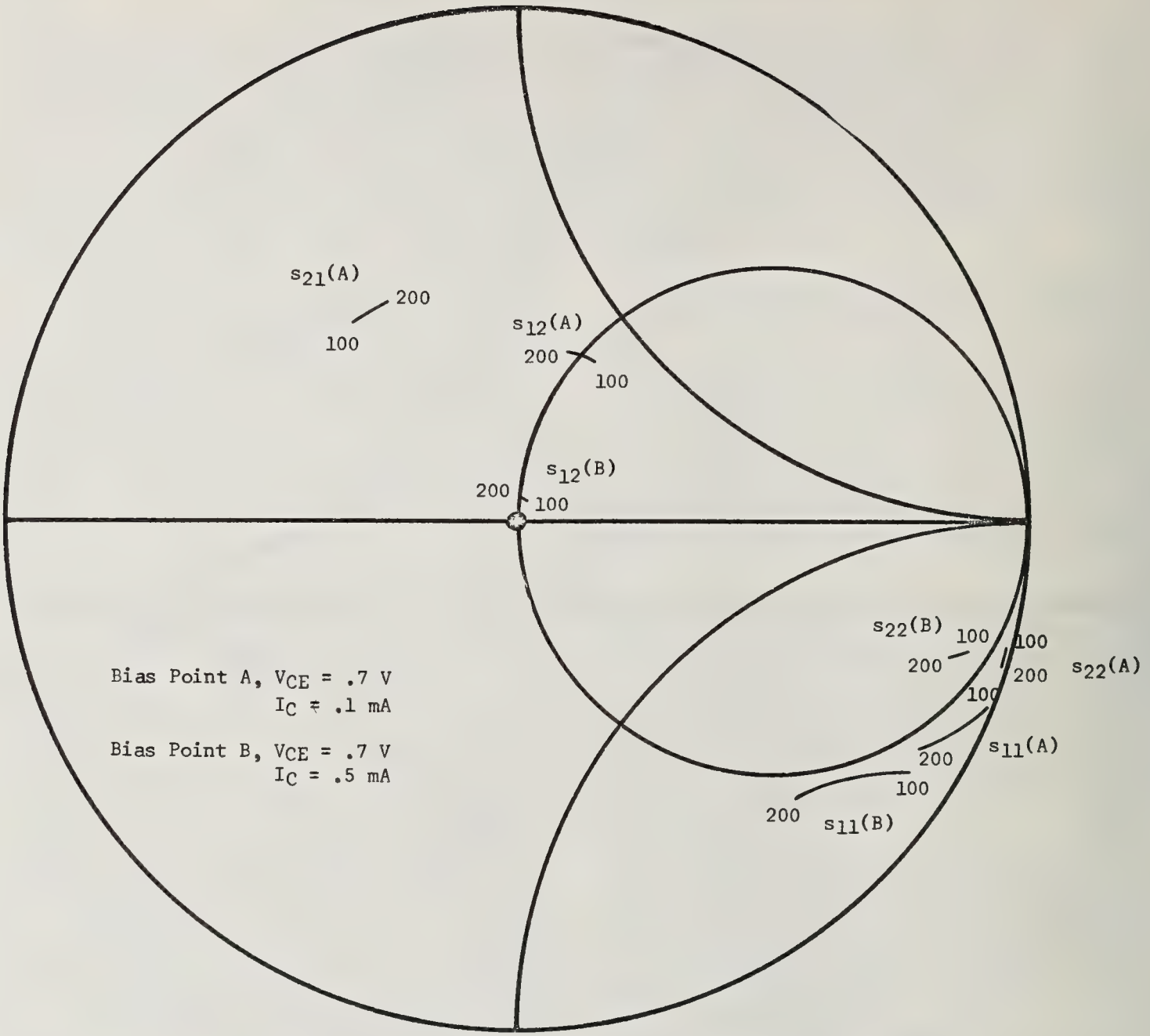


FIGURE 6.—S parameters for a transistor biased at two different points.

of 50 ohms and shunt capacity of 5.83 pF. For this crystal the manufacturer was more specific in that he shipped measured values along with the crystal.

From these plots one must conclude that the crystal of curve B more nearly follows the expected impedance curve as predicted by theory than does the crystal of curve A. One might have been able to design a circuit using a crystal equivalent circuit and the manufacturer's sketchy data for crystal B, but would have had a difficult time designing a circuit by using the 3 pieces of information supplied with crystal A. There were other crystals received from manufacturer B that did not agree with the expected curve as well as that shown, but this particular crystal was picked to indicate that some crystals do measure as theory predicts.

One special problem in designing low energy circuits is that the drive level of the crystal must be considered. Normally, the maximum drive level is of interest and must not be exceeded or the crystal will be damaged, but for low energy circuits, as are often used in bio-

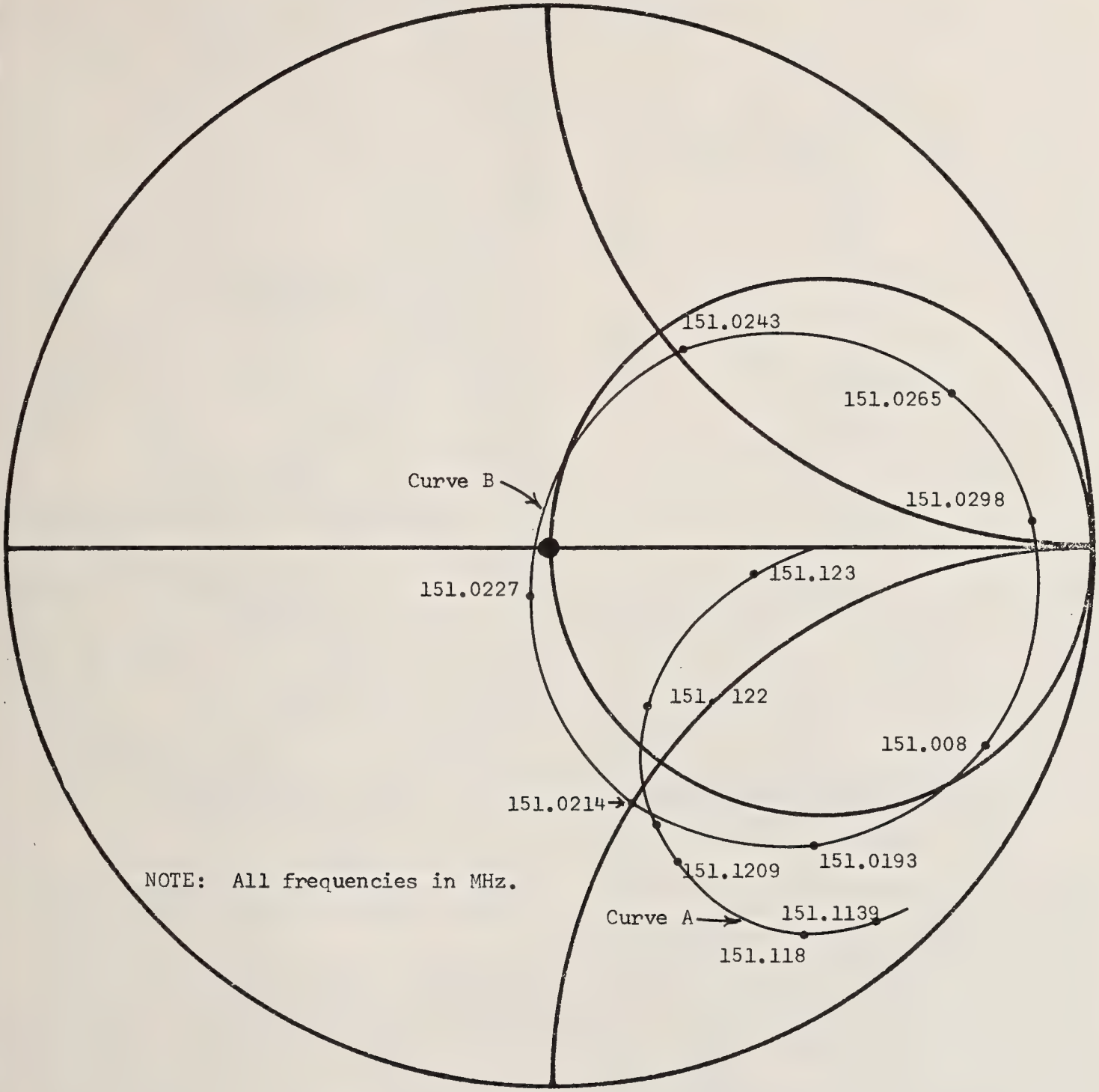


FIGURE 7.—Curves for crystals from two manufacturers.

telemetry, the minimum drive level is significant. Data were taken where the crystal did not have sufficient drive to affect in phase mechanical motion and a rather strange curve resulted. The curve is plotted in Figure 8.

The piezoelectric crystalline structure itself should not exhibit such characteristics, so one can assume that the electrode-crystal interface or the electrode size disrupts the usual well-ordered crystalline activity. This detrimental effect is especially significant at low energies.

ILLUSTRATIVE DESIGN EXAMPLE

For new designs there are always criteria set forth which must be met in order to satisfy

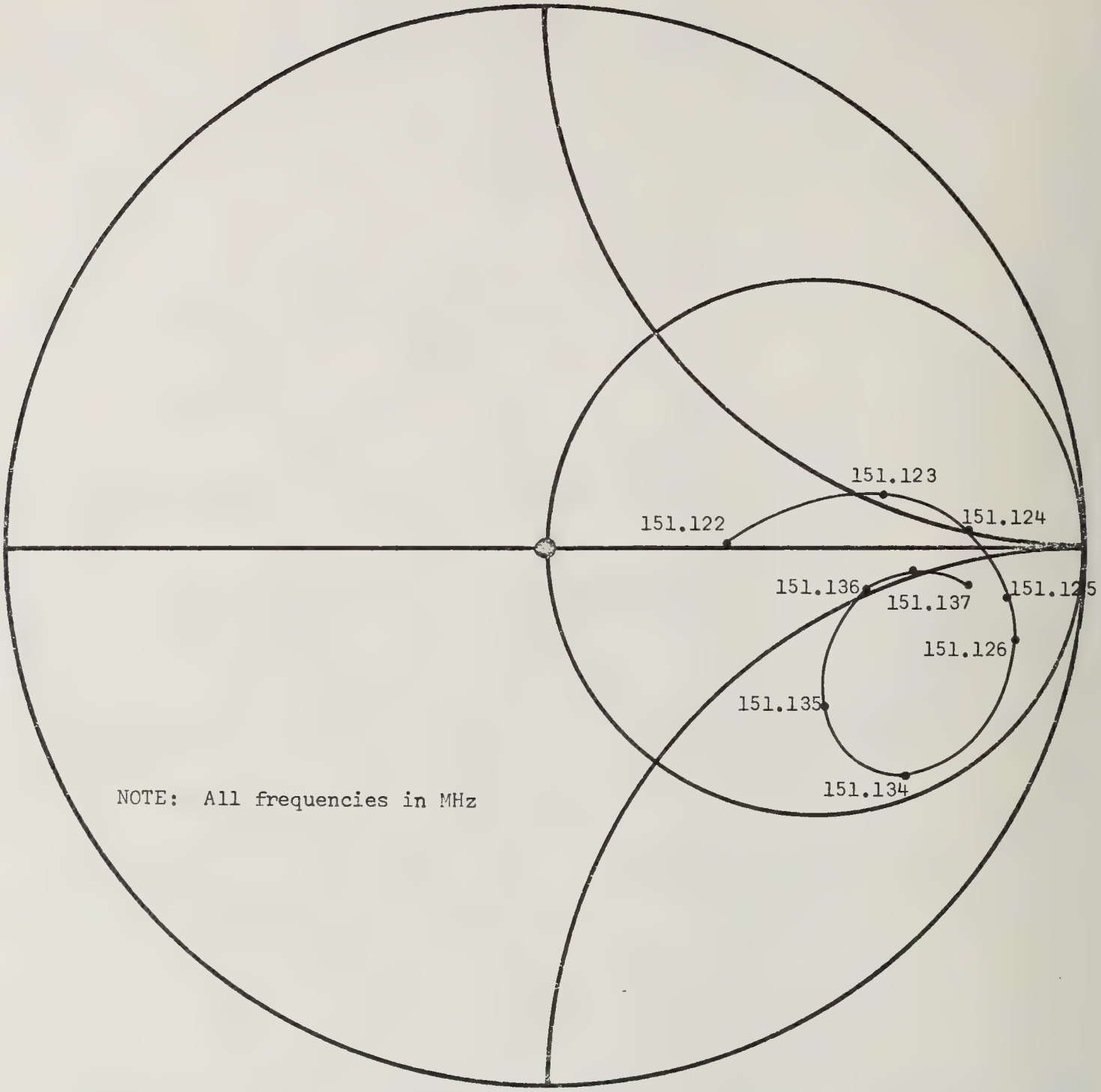


FIGURE 8.—Plot of a crystal that has insufficient drive.

the performance requirements of the complete telemetry system. For transmission from small animals and for implantation, battery size and power capability as related to current drain and longevity are pertinent factors that restrict the designer's use of many familiar circuits. More imagination has to be utilized in designing and assembling a realizable high performance system.

This design example describes a circuit that is employed for pheasant research by the Wildlife and Fisheries Sciences Department at South Dakota State University. The experiment required that there be simultaneous transmission

from several birds and that the transmission distance be one mile. Frequency spaced, continuous wave transmitters were used to accomplish the project transmitter requirements.

Crystal controlled, continuous wave transmitters were designed to operate at 151.000 MHz and above. For simultaneous use on several subjects, transmitters were frequency spaced at 10 kHz increments above 151.000 MHz. There are several advantages in adopting such a scheme. All the transmitted energy for a continuous wave transmitter is contained in a single frequency component and not spread over a frequency band. Receiver bandwidth can then be quite narrow, for noise rejection, but still have the capability to receive that energy in the single component transmitted by each transmitter. Conversely, side-bands for a pulsing transmitter can consume energy that is never received and they are especially objectionable when the transmitter is close to the receiving antenna because several frequency bands are swamped out by side-bands. With birds in the wild there isn't any sure control over their daily movements and no restriction on roosting or nesting areas. They are free to roost or nest close to, or far from, the fixed position receiving antenna. For a continuous wave system no particular problem occurs. It is true that the BFO (beat frequency oscillator) signal from CW transmission can be disguised by other extraneous signals but where there is relatively little radio activity in the 151 MHz range no difficulty is encountered.

A sensitive receiving system is obtained by employing a high gain antenna preamplifier (50 dB) that is coupled to a directional receiving antenna. The bandwidth of the receiving system is 1 MHz.

There is not a wide variety of transmitting antennas that can be used with pheasants because of their rather small size. A whip antenna seemed to function the best and was selected. The birds are not hindered in their movements by carrying a small spring-steel whip and the spring-steel flexes freely as the bird moves about. The output port of the active circuit of Figure 4 is terminated with the whip antenna and the design proceeds accordingly.

A single transistor Hartley oscillator circuit

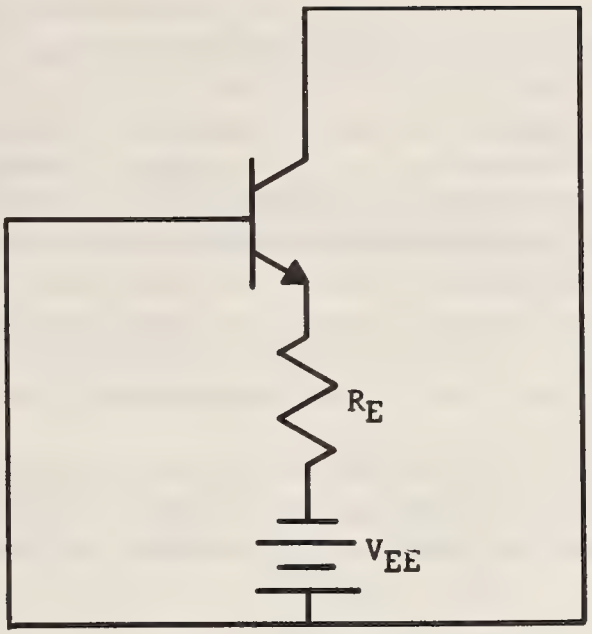


FIGURE 9.—Emitter-biasing for the Hartley oscillator.

was chosen for the active circuit. The Hartley oscillator permitted emitter biasing. This is an important feature in the design since the transmitter is used outside the year around in South Dakota where the average temperature is pleasant, but there can be extremes of —30°F to 100°F from winter to summer. The leakage current stability factor for an emitter-biased circuit can be, and for this application, is made equal to 1. Figure 9 shows the dc circuit.

Dypre [6] has shown that for a 1-port network with $s_A > 1$ the circuit will oscillate when properly terminated. Figure 10 illustrates how two networks are coupled together giving a composite circuit that will oscillate. The gain and phase criteria for oscillation are met when the active network with reflection coefficient s_A is terminated by a passive network that has a reflection coefficient, sp, equal to the reciprocal of that for the active circuit. It is assumed, and

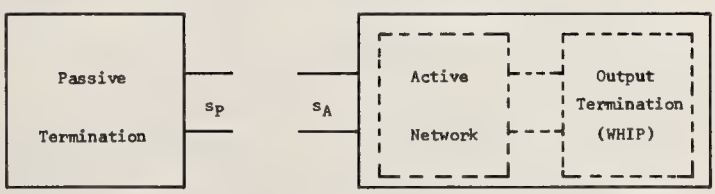


FIGURE 10.—Combination of one-port networks to make an oscillator.

is a condition for oscillation, that the reflection coefficient of the active circuit be greater than 1.

In practice the way to accomplish this at a single frequency is: (1) combine the active circuit and the ship antenna into a 1-port network, the active network of Figure 11 (notice that a matching network has been used); (2) adjust the coils, L_1 and L_2, of the active circuit until $S_A > 1$; (3) terminate with a passive network that has a reflection coefficient of $Sp = 1/S_A$.

The active and passive circuits are functions of frequency and must be handled accordingly. Where the circuits are functions of frequency, the design procedure is to plot the reciprocal of the active circuit's reflection coefficient on the Smith chart. This plot is called the active circuit contour. If the active circuit is terminated with those passive impedances as read from the Smith chart along the active circuit contour then the conditions for oscillation will be satisfied. Furthermore, oscillations will occur at the frequency read from the active circuit contour.

If a termination other than one exactly on the active circuit contour is used, the circuit will do one of two things: (1) oscillate and overdrive or (2) fail to oscillate. Basically, the active

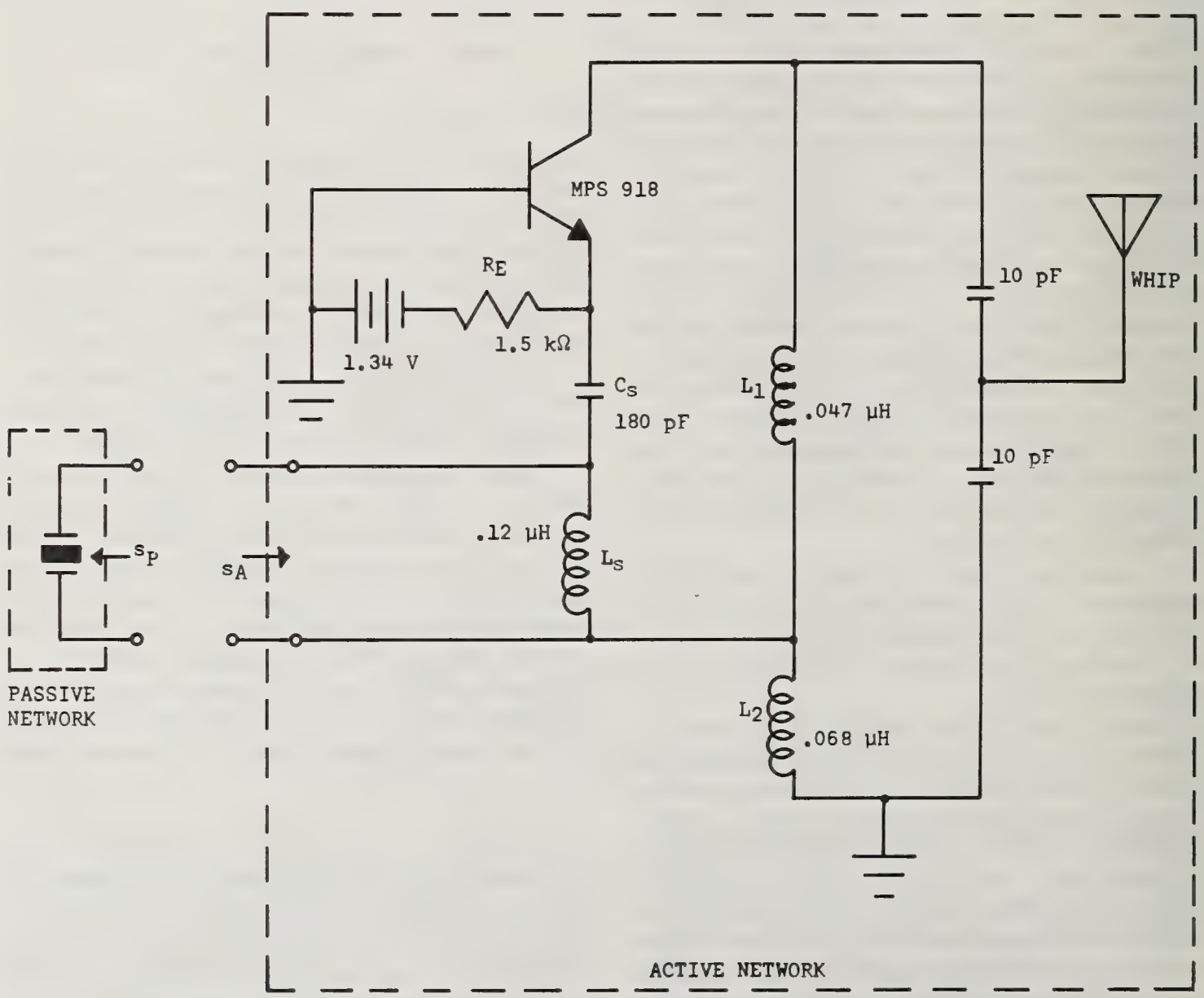


FIGURE 11.—Transmitter circuit.

circuit contour is the boundary between a stable or an unstable circuit.

Now, continuing with our example a crystal controlled oscillator was required so the passive network at the input must be a crystal. A plot for a typical crystal was given in Figure 7, curve B, and is repeated in Figure 12 for clarity. The designer's termination options have now been exhausted because the input and output terminations have both been specified, and the difficult task of designing the active circuit remains.

There isn't any step-by-step procedure applicable to the design of the active circuit. How-

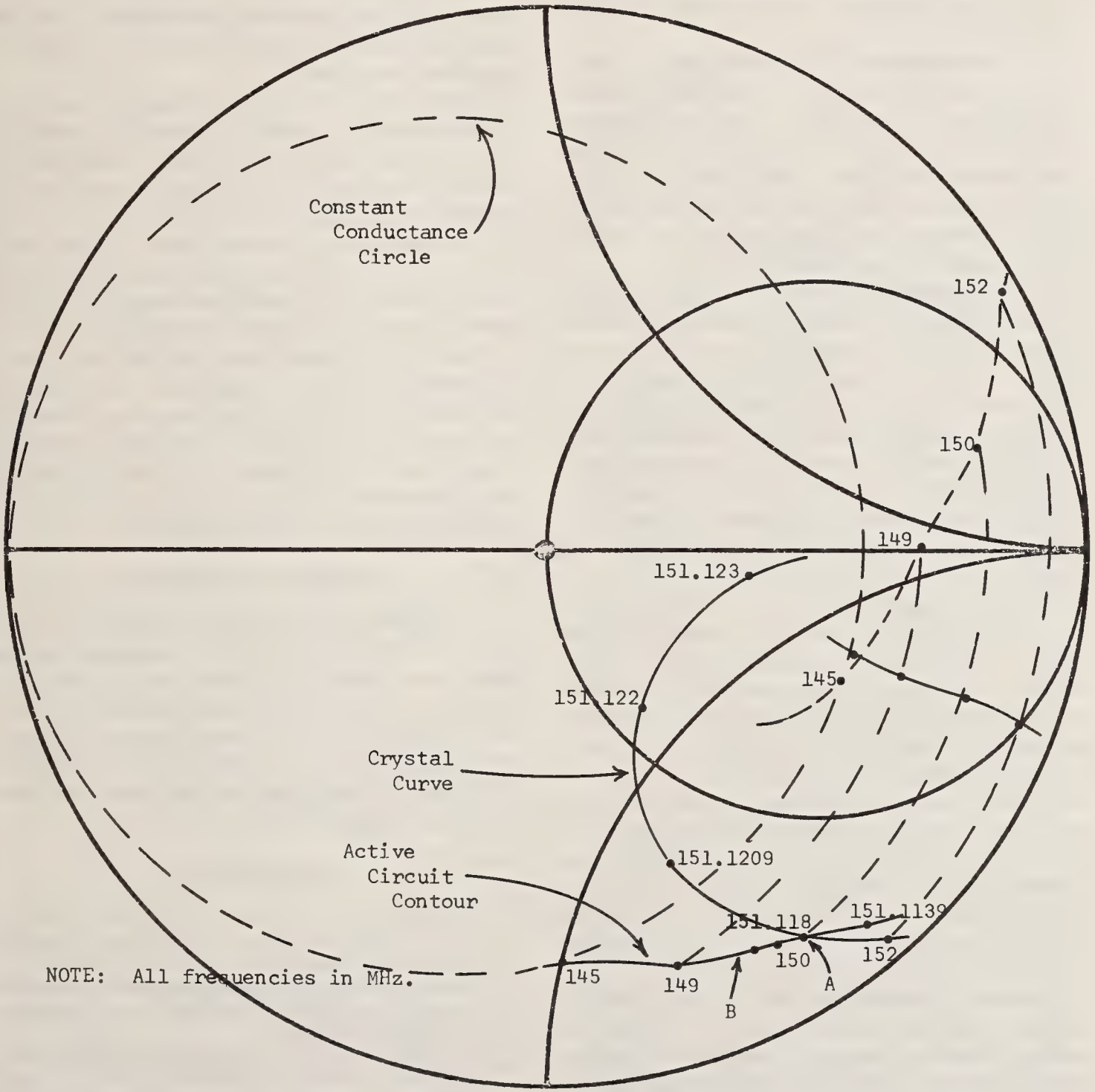


FIGURE 12.—Active and passive circuits combined to make an oscillator. (f = 151.118 MHz) Dashed curves show effects of shunt inductor.

ever, some general rules help. Tuning of the tank circuit in the Hartley oscillator may not change the active circuit contour significantly but does change the frequency relationship of the contour. For example, a change in tank tuning moves point A, Figure 12, which corresponds to a frequency of 151.118 MHz to position B. In this fashion retuning gives the option of moving a particular frequency to a desired point on the contour.

There is another way of significantly controlling the contour and that is by adding shunt elements across the terminals of the active device. A shunt inductor rotates the contour clockwise along paths of constant conductance. A shunt capacitor rotates the contour counter-clockwise along the same paths. This operation is depicted by the dashed curve in Figure 12.

The method of retuning and adding shunt elements allows for a degree of maneuverability of the active circuit contour. This control is not as well defined as one would like because as components are added, or resonant frequencies changed, the quality factors (Q) controlling the circuit also change. These non-ideal components cause deviations from theoretically "nice" results, but with a little experience it is not difficult to become adept at "manipulating contours."

All that remains now is to manipulate the active circuit contour until it is tangent to the crystal contour at a particular point. The inductor L_s, as shown in the complete circuit diagram of Figure 11, situates the contours so they are tangent. Since the crystal curve is a function of frequency, every point on the curve corresponds to a unique frequency; thus, the tangent point relates directly to the frequency of oscillation, 151.118 MHz. Note that this frequency does not necessarily have to be the series resonant frequency of the crystal. Capacitor C_S in the schematic diagram is a dc blocking element.

Certainly, it would be unfair to leave the impression that components must be lumped together into only two ports, active and passive, before a design can be completed. From the fact that the s-parameters, or any other convertible set of parameters, are exactly known, a system can be mathematically designed that will function; however, so far there isn't any set of design rules that automatically leads one through the design process. Certain experience, even though based on classical circuit design techniques, is still required.

MODULATION CIRCUITS

There are two active circuit contours that show how FM (frequency modulation) can be obtained by using a purely resistive or a purely reactive transducer.

Figure 13 shows the two cases. Case I illustrates an active circuit contour the frequency of which can be controlled by a resistance. A change from point A, 70 ohms to point B, 100 ohms forces a change in frequency of 10 MHz. By design, a contour such as Case II of the same figure can be obtained where a change of 10 MHz is caused by a reactive change of from 10 ohms at point C to 50 ohms at point D.

This design method requires that one know the relationship of the transducer reflection coefficient over the frequency range of interest, and then, for a specified Δf of the modulator, design the active circuit. The design method for FM systems is not much different from that discussed in the preceding example.

ACKNOWLEDGEMENTS

A practical data acquisition system has been designed where the methods presented in this paper were used. The project was a joint endeavor between the Electrical Engineering and the Wildlife and Fisheries Sciences Departments (S–521) at South Dakota State University. Much of the electronic research was supported by NSF Grant GK–21034.

REFERENCES

1. Bodway, George. Two port flow analysis using generalized scattering parameters. Microwave Journal, Vol. 10, No. 6, May 1967.
2. Weinert, Fritz. Scattering parameters speed design of high-frequency transistor circuits. Electronics, September 6, 1966.
3. Froehner, William. Quick amplifier design with scattering parameters. Electronics, October 16, 1967.
4. Anderson, Richard. S-parameter techniques for faster, more accurate network design. Hewlett-Packard Journal, Vol. 18, No. 6, February 1969.
5. Kim, Young D. Scattering parameters of VHF

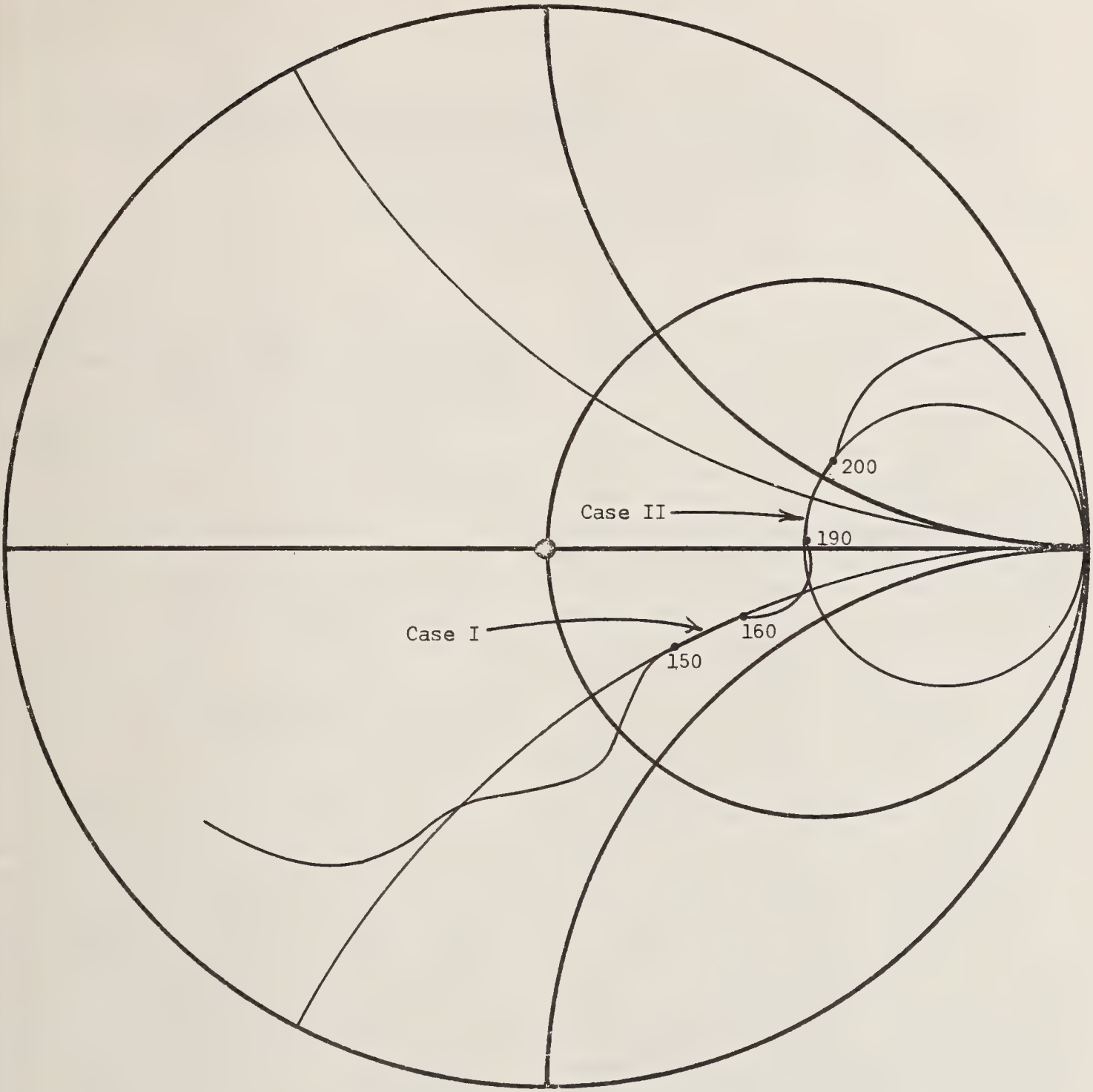


FIGURE 13.—FM modulator using ΔR or ΔX.

semiconductor devices. M.S. thesis, South Dakota State University, pp. 52–66, 126–129, 1970.

6. DYPRE, J. and BODWAY, GEORGE. The application of scattering parameters to high frequency circuit design. IEEE Intntl Conv. Digest, New York, 1970.

DISCUSSION

QUESTIONER (unidentified): You mentioned 150 to 160 MHz. You were talking about crystals there, or were you talking about other transducers?

DR. ELLERBRUCH: No, you see when I talked about that (Figure 13), I would take the crystal out. But with a crystal in that range on a seventh overtone, you can drive the crystal maybe 5, 10, 15 kHz.

MEASUREMENT AND RADIOTELEMETRY OF CARDIOVASCULAR VARIABLES IN CONSCIOUS ANIMALS: TECHNIQUES AND APPLICATIONS

Dean Franklin, Stephen F. Vatner, Charles B. Higgins,
Thomas Patrick, W. Scott Kemper and Robert L. Van Citters*

Techniques: Among the primary impediments to verification of hypotheses regarding the extent to which identified cardiovascular control mechanisms are brought into play in the normal animal is the inability to measure the fundamental variables in the normal animal undergoing spontaneous activities. To this end we have developed techniques suitable for measurement and radiotelemetry of the basic hemodynamic variables in untethered, conscious animals. The system provides the capability for measurement of ventricular and arterial pressures, regional blood flows (coronary, mesenteric, renal and iliac), and of ventricular and aortic dimensions. The system adequacy has been proved in successful applications ranging from studies of cardiovascular dynamics in exercising sled dogs to studies of giraffes and baboons in habitat.

Applications: Ideally, cardiovascular control mechanisms should be elucidated in man. Unfortunately, the human cardiovascular physiologist has markedly limited access to measurement of the fundamental circulatory variable on the basis that atraumatic, harmless, instrumentation techniques for measurement of these variables has not yet been developed. For these reasons cardiovascular physiology and pharmacology are most commonly studied in animal models of man.

Accordingly, we have developed instrumentation and handling techniques to directly measure and radiotelemeter measurements of blood flows and pressures and cardiac dimensions from healthy, conscious untethered animals. We have applied these techniques to a wide variety of experimental situations including elucidating cardiovascular physiological and pharmacological control mechanisms in conscious animals, studying coronary dynamics in untethered baboons, blood pressure responses in free-ranging giraffes and regional blood flow distribution during severe spontaneous exercise in dogs unencumbered by tethers, treadmills and the laboratory environment.

INTRODUCTION**

A major fraction of our knowledge of human cardiovascular physiology is derived from animal models with the assumption that knowledge so derived is applicable to humans. For example, a basic cardiovascular control mechanism identified by experiments in dogs is assumed to have its counterpart in the human cardiovascular system. Indeed, it is rare when results from animal experiments are totally inapplicable to humans. However, most commonly such experiments are conducted using anesthetized animals or excised tissue. While such experiments reveal mechanisms by which the cardiovascular system is controlled, they do not confirm the extent to which so identified mechanisms are drawn into play in the intact, unanesthetized mammal. For example, a potent neural mechanism capable of reducing kidney blood flow to zero, has been repeatedly identified in anesthetized animal preparations. This mechanism traditionally has been believed to reduce blood flow to the kidney during conditions of stress such as extreme exercise in the normal animal, i.e., blood flow was thought to be shunted from the kidney to provide a part of the additional flow required by the exercising muscle. Recent work using direct measurements of renal blood flow during extreme exercise in dogs has demonstrated that flow to the kidney does not decrease during exercise except under the most unusual (and probably pathological) circumstances.

It is clear that hypotheses regarding cardiovascular function and control must be ultimately tested in normal, conscious animals with all control mechanisms intact and under as nearly normal conditions as possible. To this end we have developed an instrumentation system suitable for measurement and radiotelemetry of fundamental cardiovascular mechanical variables in animals undergoing spontaneous activities. The three variables used to describe gross hemo-

* Department of Medicine, University of California, San Diego, Scripps Clinic and Research Foundation, La Jolla, California, 92037, and University of Washington, Seattle, Washington.

** Supported in part by U. S. Public Health Service Grant Nos. HL 12373 and HL 13441.

dynamics in chronic animal preparations are regional blood flows, ventricular and aortic pressures, and ventricular dimensions. In addition, an interrogation system is carried by the animal to provide the capability for remote control of power, selection of measuring sites, etc. Each of these subsystems will be described individually.

TECHNIQUES

Flow Measurement

Regional blood flows are measured using the continuous wave ultrasonic Doppler shift blood flowmeter. The system utilizes the Doppler principle whereby sound reflected from a moving target will be shifted in frequency by an amount proportional to the target velocity. For blood flow measurement, 9 MHz sound is beamed through the unopened blood vessel wall into the bloodstream. The sound is scattered isotropically, primarily by the erythrocytes, and a fraction of the backscattered sound travels through the blood vessel wall where it is sensed. The backscattered sound differs in frequency from the incident sound by an amount proportional to the blood velocity, in accordance with the Doppler equation which states that the shift in frequency equals:

$$\frac{2 F_t \quad v \cos \alpha}{C}$$

where F_t = incident sound frequency
v = erythrocyte velocity
α = angle between the sound axis and the flow axis
c = velocity of sound in blood

For a practical transducer operating at 10 MHz the Doppler shift in frequency will be nominally 100 Hz/cm/sec. The backscattered signal is mixed with the projected signal to produce the sum and difference frequency signals. The frequency of the difference frequency signal is recorded and calibrated in terms of instantaneous blood velocity. So long as the cuff-like transducer constrains the blood vessel to a constant diameter, the blood velocity as measured by the Doppler flowmeter provides an accurate measure of volume flow.

The component parts of the flowmeter are the transducer which is implanted around the blood vessel, an oscillator to provide 9 MHz power to excite the piezoelectric crystal within the transducer, and the receiver which amplifies and demodulates the backscattered sound signal sensed by the transducer.

Transducers

The implantable transducer has the form of two molded expandable polystyrene hemicylinders hinged together to form a cylinder which is clamped around the exposed blood vessel. A piezoelectric plate (LTZ–5, lead zirconate-titanate, 10 MHz) is glued within each half of the cylinder. The plates are angled at 45° with the flow axis so that the acoustical fields of the plates intersect within the lumen of the cylindrical transducer, i.e., in the center of the bloodstream. PVC insulated, multistrand, copper wires in twisted pairs are connected to the electrodes of the crystals. The low impedance of the crystals (50–200 ohms at resonance) along with the inherent frequency modulation operating principle makes the Doppler flow system relatively insensitive to environmental noise and electrical shunting of tissue fluids. Thus, extreme electrical insulation and shielding are not required.

At operation, the blood vessel is exposed and the transducer is tied in position around the blood vessel. The wires are passed subcutaneously to the area between the scapulae and the wires brought through the skin. The transducer material prompts a transient inflammatory response with resultant growth of connective tissue from the blood vessel around the transducer and also a growth of tissue up into the cavity between the angled crystal plate and the wall of the blood vessel. The connective tissue in contact with the crystal forms an acoustical path for the sound to travel from the crystal into the blood vessel. After approximately a week the tissue is adherent to the transducer body, and the transducer can be expected to function routinely for three months. The most common failure mode is corrosion and breakage of the copper wires. Where experiments are to be conducted for longer periods of time, stranded stainless steel wire should be used instead of the copper wire.

Exciter

Typically, an electrical signal of approximately 3 v peak to peak at the resonant frequency of the crystal is used to generate the 10 MHz sound beam. The crystal material has an extremely high dielectric constant such that a practical transducer may have a shunt capacitance as high as 10^{-9} farads. At resonance the resistive component of the impedance is on the order of 100 ohms. Thus, the crystal exciter must be capable of producing a 10 MHz signal with an output power on the order of 20 mw into an impedance on the order of 100 ohms. Variable acoustical standing waves will be generated by the crystal-transducer when implanted, such that the impedance of the projector crystal will vary. This varying impedance can be reflected back to the electrical generator and modify the frequency of the oscillator. This varying frequency signal will be indistinguishable from a Doppler shift so that provision for buffering the crystal from the oscillator should be provided to minimize spurious measurements. With these considerations in mind, a low power, 2 stage exciter is usually used. This consists of an oscillator, variable in frequency from 7–10 MHz, followed by a buffer amplifier stage which is matched to the impedance of the crystal.

Receiver

The electrical signal at the receiver crystal consists of two components. One component is due to the direct coupling of sound from the projector crystal across the blood vessel to the receiver crystal. This signal is unmodulated, i.e., it is at the same frequency as the projected signal frequency, and is relatively high in amplitude, i.e., 1–10 mv. The second signal is the signal backscattered from the moving erythrocytes. This signal is modulated in frequency in proportion to the velocity of the moving erythrocytes. Its amplitude is relatively small, i.e., 1–30 μv. The receiver crystal is identical to the projector crystal and appears electrically as a generator with approximately a 50 ohm source impedance. The first stage of the receiver is then a low-noise radiofrequency amplifier with a gain of 30 and with an input impedance of approximately 50 ohms. The amplified composite radiofrequency signal is then passed through a mixer to generate an audiofrequency signal with a frequency equal to the difference in frequency between the large directly coupled signal and the signal backscattered from the erythrocytes. A power detector is used for this mixing and audio amplification. The resultant signal is filtered to eliminate the 10 MHz carrier signal and the sum frequency signal. The output of the receiver is typically a 30 mv peak to peak signal varying in frequency from 0–10 kHz as the blood velocity varies from 0–100 cm/sec. In other words, a frequency modulated, audio frequency signal is produced, the frequency of which is proportional to the flow of blood through the implanted transducer.

Pressure Measurement

Pressure is measured by the chronic implantation of a miniature pressure gauge within the apical lumen of the left ventricle or within the lumen of the thoracic or abdominal aorta. The pressure gauge is a disc-like structure 6 mm in diameter x 2 mm in thickness with the wires exiting from the back of the disc. The front of the disc is formed by a thin titanium diaphragm on the back of which are deposited four silicon strain gauges connected electrically in the form of a wheatstone bridge. As the diaphragm is deformed by pressure, the strain gauges are stressed and their electrical resistance changes in proportion to the pressure applied to the metal diaphragm. The voltage output from the gauge, when excited with 5.6 v DC, varies from 0 to 45 mv as the applied pressure varies from 0 to 300 mm Hg. The gauge is resistively temperature compensated, such that the zero baseline and sensitivity are independent of temperature over a wide range (less than 0.5 mm Hg/°C).

In practice, the power for excitation of the bridge is provided by an 8 v battery. The output of the battery is voltage regulated to provide a constant 5.6 volts to the bridge. The voltage from the pressure gauge is amplified to provide 0.3 volts full scale output.

The gauge is calibrated prior to implantation, but this calibration is used only as a guide and

to monitor the condition of the pressure gauge. The drift rate of the gauges when implanted is significant for chronic applications. A small fraction of the gauges drift insignificantly over a period of a month while another small fraction drift as much as 50% of full scale over a period of a month. The drift rate is different after implantation than when they are stored. The typical zero baseline drift rate after implantation is on the order of 10 mm Hg/month. The sensitivity of the gauges also decreases with time, typically on the order of 5%/month. For these reasons in vivo calibration using a catheter is essential for accurate pressure monitoring. The frequency response of the pressure gauges is well above that required for reproduction of the high frequency components of the pressure waveforms. Clotting caused by the gauge is a rare but real problem. Occasional massive embolization will occur. Necrosis of the tissue around the pressure gauge and the associated sutures with resultant rupture of the aorta or ventricle is an occasional problem. With all of these problems, implantation of the miniature gauge is still probably the best compromise for provision of a high fidelity recording of continuous blood pressure in a form suitable for radiotelemetry.

Dimension Measurement

The transit time of sound travelling between two crystals sutured in opposition across the left ventricle is taken as a measure of the diameter of the left ventricle. The transducer consists of piezoelectric discs (LTZ–2, 5 MHz, 3 mm x 0.5 mm approx.) with a styrene diverging lens glued to one side of the disc. The crystals are backed with a dacron patch epoxied to the crystal so that sutures can pass through the dacron to fasten the transducer to the epicardium. PVC insulated, twisted pairs of multistrand copper wire are soldered to the electrodes on the crystals. For measurement of ventricular diameter the two crystals are sutured to the epicardium in the area of the interventricular sulci opposing each other across a diameter of the left ventricle. The wires are passed subcutaneously to the area of the scapulae where they are brought through the skin.

A brief (.2 μsec) electrical pulse is applied to one crystal. It responds by vibrating transiently and this acoustical pulse is coupled into the epicardium and travels across the ventricle to strike the other crystal. The electrical signal occurring across the receiver crystal when the sound strikes it is amplified to form an electrical pulse delayed in time from the initial pulse by an amount equal to the transit time of the sound. A rectangular waveform of voltage is generated which begins when the sound begins its transit and which is terminated by the sound arriving at the second crystal. This process is repeated 2000 times/sec. The resultant train of rectangular voltage waveforms is filtered (averaged) to form a voltage proportional to the transit time of sound across the ventricle. This voltage is amplified to a level appropriate for driving the telemetry apparatus. The frequency response of the system is limited electronically to 100 Hz. The minimum separation that can be measured is on the order of 5 mm. The stability of the system is typically 0.03 mm/hr drift but this drift rate can be eliminated by direct calibration on an oscilloscope against a crystal oscillator. The advantages of this approach include a light-weight, essentially inertialess transducing system for provision of an extremely accurate measurement of dimension. The primary difficulty involved in the practical application of this approach involves the orientation of the transducers. The sound beams are relatively directional and although a diverging lens is used on the crystals, some experience is required to place the crystals such that each will lie in the sound field of the other. If the crystals are not oriented into each other properly the received signal will be sufficiently low or will change so drastically during the cardiac cycle as to preclude an accurate measurement. This is quite apparent when the received signal is viewed on the oscilloscope.

Radiotelemetry

The radiotelemetry system is a straightforward adaptation of aerospace FM/FM techniques, i.e., frequency modulated subcarriers are summed and the composite signal modulates the frequency of a VHF radio transmitter.

The signal is radiated from the transmitter on the animal to a remote receiver where the original signals are separated by frequency filtering. Each of the separate frequency modulated signals are restored to their original form using frequency discrimination. The output of the discriminators are voltages proportional to the measured variables and are recorded on magnetic tape and a strip chart recorder.

The basic VHF transmitter employed is a 50 MHz oscillator with a varactor diode in the frequency determining circuitry of the oscillator. The modulating signal is coupled to the oscillator to modify the capacitance of the varactor and thus modulate the frequency of the 50 MHz oscillator. The 50 MHz signal is coupled to a second amplifier which serves as a frequency doubler and power amplifier-buffer. The 100 MHz output of this amplifier is matched into a stub antenna for short range telemetry, i.e., a few hundred feet. Where longer telemetry ranges are needed, the 100 MHz signal is multiplied to 400 MHz which allows use of a full resonant transmitting antenna with consequent increase in radiation efficiency and range (line of sight).

As was indicated above, each of the variables to be transmitted must be in the form of a relatively low frequency, frequency modulated signal. The Doppler flowmeter is unique in this manner in that the output of the Doppler flowmeter is inherently a frequency modulated, audio frequency signal. Thus, no further signal conditioning must be provided for FM/FM radiotelemetry, i.e., the output of the Doppler flowmeter can be coupled directly to the input of the frequency modulated radiotelemetry transmitter for transmission. This approach retains the inherent zero stability of the Doppler flowmeter and the signal need not be demodulated within the instrumentation carried by the animal. A separate subcarrier oscillator is provided for each of the other variables, i.e., pressure and dimension. Typically, the pressure signal is coupled to a voltage controlled oscillator operating on IRIG band #15 to produce a signal varying in frequency from 28 kHz to 32 kHz as pressure varies from 0 to 300 mm Hg. This 30 kHz FM signal is summed with the 0–15 kHz signal from the Doppler flowmeter and the composite signal is coupled to the 50 MHz oscillator within the telemetry transmitter. Alternatively, the pressure signal is coupled to a channel 15 voltage controlled oscillator and the diameter signal is coupled to a channel 13 voltage controlled oscillator and these two signals are summed to drive the telemetry transmitter.

Power and Remote Control

Power for the components carried on the animal is provided by 2000 ma hr mercury batteries (Mallory TR 236–R). Although the discharge voltage from mercury batteries is relatively constant, voltage regulators are included to provide power to the voltage sensitive components, primarily the voltage controlled oscillator and pressure gauge. Power consumption is typically 400 mw for each of the measuring instruments and 150 mw for the telemetry transmitter, so that total continuous operating time of the instruments on one set of batteries is typically 24 hours.

There are instances when continuous operation is not appropriate for an experimental situation. For example, where a baboon is to be observed intermittently for a number of days, continuous operation of the measuring instruments would require the baboon to carry an inordinate number of batteries. To overcome this difficulty, a remote control device for turning the power on and off at will is built into the system. The animal carries a miniature, low power, 72 MHz AM radio receiver which is continuously powered. The output of this radio receiver is coupled to 4, low frequency, narrow band filters. When a signal passes through one of the filters a latching relay is thrown. In this way one can switch a latching relay by transmitting a 72 MHz signal modulated by a frequency corresponding to one of the filters. The latching relay is thrown to its original position by transmitting a tone corresponding to one of the other filters connected to switch the relay. In other words, one can control 4 functions by transmitting the 4 tones corresponding to the frequency of the 4 filters. This remote control capability has advantages beyond simple control of power. The animals are typically instrumented with 3 flow transducers at three differ-

ent sites of interest. These transducers can be connected through electromechanical relays to a single flow meter. By switching among the three flow transducers one can sequentially sample 3 different flows without approaching the animal for resoldering of wires. This has proved to be a considerable time-saving convenience, particularly when intractable animals, such as baboons, are being studied. In addition, the remote control feature provides a capability for electrical stimulation or infusion of cardioactive drugs.

The receiving station consists of an appropriate receiving antenna, FM radio receiver, appropriate discriminators and a magnetic tape recorder for storage of the information. For field operation this equipment is housed within a truck to serve as a mobile receiving station. This approach is particularly effective for studies of exercising dogs where the animal runs spontaneously behind the moving recording vehicle.

The receiving antenna is a standard entertainment quality FM 100 MHz 5 element Yagi antenna.

The 100 MHz signal from the antenna is coupled to an entertainment quality FM radio. The deemphasis network is bypassed in the radio receiver to provide a linear frequency response. The output of the radio receiver is coupled to appropriate frequency discriminators. Where voltage control oscillators are used on the animal these are standard commercially available frequency-to-voltage converters. The Doppler flow signal requires a special kind of discriminator. While in principle it is similar to the standard discriminators, the frequency deviation is so wide in the Doppler flow signal that a special discriminator is required. The practical instrument currently in use provides a voltage proportional to the average number of zero crossings per unit time of the input signal over a frequency range of from 200 Hz to 20 kHz. The output frequency response of the discriminators is limited electronically to 100 Hz.

APPLICATIONS

While the capability for measurement of blood flows, pressures and cardiac dimensions in conscious animals has extended the scope of cardiovascular physiological investigation, the ability to monitor these variables remotely has further extended the capabilities of the physiologist. There are certain studies for which telemetry is essential, for instance, studies conducted outside the confines of the laboratory and especially the study of animals in their natural habitat. Other studies, which involve non docile animals, such as the larger primates, are also made feasible through the use of telemetry techniques. In addition it is apparent that any study in conscious animals is influenced to an unknown extent and may even be limited by the laboratory environment. Several of these studies have been demonstrated to be improved substantially by applications of telemetry techniques. For instance, the study of maximal exercise in dogs is difficult if not impossible utilizing "directly wired" techniques and treadmill exercise. However, it is not uncommon for dogs to run spontaneously at speeds of greater than 20 miles/hour in the field. The capability for telemetry provides a means by which a description of the cardiovascular response to severe exercise can be accomplished.

The system developed for radiotelemetry of left ventricular dimensions and pressure has been described in more detail previously.[1,2] In brief, an improved ultrasonic dimension gauge was used, which measures the transit time of acoustic impulses travelling at the sonic velocity of approximately 1.5×10^6 mm/sec between the two 5-MHz piezoelectric crystals sutured in opposition on the epicardial surfaces of the left ventricle. A voltage proportional to transit time is recorded and calibrated in terms of crystal separation and thus provides a continuous measurement of ventricular size. The signals from the diameter gauges and pressure implant system are transmitted from the animals via a two-channel FM–FM telemetry system to the mobile recording unit. A measure of left ventricular pressure is provided by the miniature pressure gauge[3] implanted within the left ventricle. A voltage proportional to pressure is obtained, which after appropriate amplification, can be telemetered along with a measure of dimensions using separate voltage-controlled oscillators.

The system for measurement and telemetry of blood flow using the ultrasonic Doppler telemetry flowmeter has also been described in detail.[2,4–7] In brief, the Doppler flow signal with frequency components from 200 Hz to 15 kHz, is linearly summed with the voltage-controlled oscillator signal representing pressure to form a frequency-multiplexed video signal. This signal is applied to the input of a frequency-modulated, radio frequency transmitter operating in the 88–108 MHz range. The power output, approximately 10 mw, coupled to a shortened, inductively, loaded stub antenna, is adequate to transmit the signals over a several hundred foot range to the mobile recording station.

The mobile recording unit consists of an FM communications receiver, subcarrier discriminator, signal processing electronics, and a tape recorder. A commercial 5-element FM antenna, mounted on the roof of the van, serves to receive the telemetered signals from the animal. Power for the equipment is obtained from a DC to AC inverter (Heathkit Model MP–14, Heathkit, Benton Harbor, Mich.) which is powered by a 12-volt automobile battery.

The FM tuner (Heathkit Model AJ–15), modified to disable the FM deemphasis network and multiplex circuitry, provides video response sufficient to recover both the Doppler flow signal (200 Hz–15 kHz) and the 37–43 kHz pressure-modulated subcarrier signal. The output of the FM tuner is processed by a 15 kHz low-pass filter to extract the Doppler flow signal which is then recorded on one channel of the tape recorder (Honeywell Model 5600, Honeywell, Minneapolis, Min.). The output of the tuner is also applied directly to the input of a subcarrier frequency discriminator (Airpax Model FDS–30, Airpax Electronics, Inc.) which converts the 37 to 43 kHz frequency modulation signal to a DC voltage proportional to pressure, which is coupled to another channel of the tape recorder.

Exercise studies conducted in the field with dogs running spontaneously at speeds of greater than 20 miles/hour are superior to treadmill exercise not only because they yield a purer form of exercise with less components of excitement, but also the level of exercise attained is greater. Thus, control mechanisms which can only be elicited with severe exertion might not be evident with the moderate exercise attained on the treadmill. An example of this is the left ventricular response to exertion. Studies conducted in dogs running on the treadmill indicated that the left ventricular response to exertion occurred almost entirely through increases in heart rate;[8,9] whereas, the contribution of the Frank-Starling mechanism, a means by which the heart can augment its output by increasing initial myocardial fiber length, was not evident.[8,9] We recently re-examined this problem in conscious untethered dogs running spontaneously in the field at speeds of greater than 20 miles/hour. In this study, measurements of left ventricular dimensions and pressure were telemetered to a mobile recording unit. The results of this study indicated that during severe exertion near maximal increases in heart rate (over 300 beats/min) and contractility (dP/dt over 12,000 mm Hg/sec) occurred, while in all eight animals studied a significant increase in left ventricular end diastolic dimensions could be identified.[10] Thus, by utilizing radiotelemetry techniques to study severe exercise, it was found that an important cardiovascular control mechanism (Frank-Starling) is utilized only in situations of extreme stress.

We have applied the techniques for radiotelemetry of simultaneous measurements of blood flow and arterial pressure to describe the normal and abnormal peripheral circulatory adjustments to severe exercise. It is traditionally held that exercise causes sufficient increases in visceral vascular resistance to reduce visceral flow in order to provide additional flow to the active musculature.[11–18] However, treadmill exercise in dogs failed to produce significant reductions in visceral flow.[19,20] It was demonstrated[10] that treadmill exercise is not sufficiently severe to elicit this circulatory compensatory mechanism. Accordingly, measurements of visceral flows and arterial pressure were telemetered from Alaska sled dogs running great distances in sub-zero weather[21] and also from mongrel dogs running spontaneously in the field at speeds greater than 20 miles/hour.[7] This level of exercise was sufficiently severe to elevate heart rate to 300/min in some dogs and to increase mean arterial pressure from 89 to 140



FIGURE 1.—An instrumented dog running behind the mobile recording unit at a speed of 22 mph. Signals containing the flow/pressure measurements are telemetered from the apparatus carried by the dog in saddlebags. The FM signal is received by the antenna on the van and is coupled to the recording instruments inside. (Reproduced with permission from J. Clin. Invest. 50:1950, 1971).

mm Hg [7] and to quadruple coronary blood flow.[22] Although resistance increased substantially in the mesenteric and renal beds during exercise, both mesenteric and renal flows remained essentially constant [7] (Figure 1). Thus, even near maximal exercise was not sufficient to invoke the circulatory control mechanism of reduction and diversion of visceral flow.

Even though diversion of visceral flow during exercise could not be demonstrated in normal dogs, it was considered possible that this compensatory mechanism is utilized when the normal primary adjustments to exercise are impaired. We evaluated this hypothesis systematically by examining the response to severe exercise in dogs with induced pathological impairments limiting either increases in heart rate or stroke volume or the oxygen-carrying capacity of the blood. Changes in heart rate were limited in a series of dogs by surgical induction of chronic complete heart block.[7] Stroke volume was limited by induction of chronic experimental heart failure (tricuspid avulsion and progressive pulmonary stenosis) in another

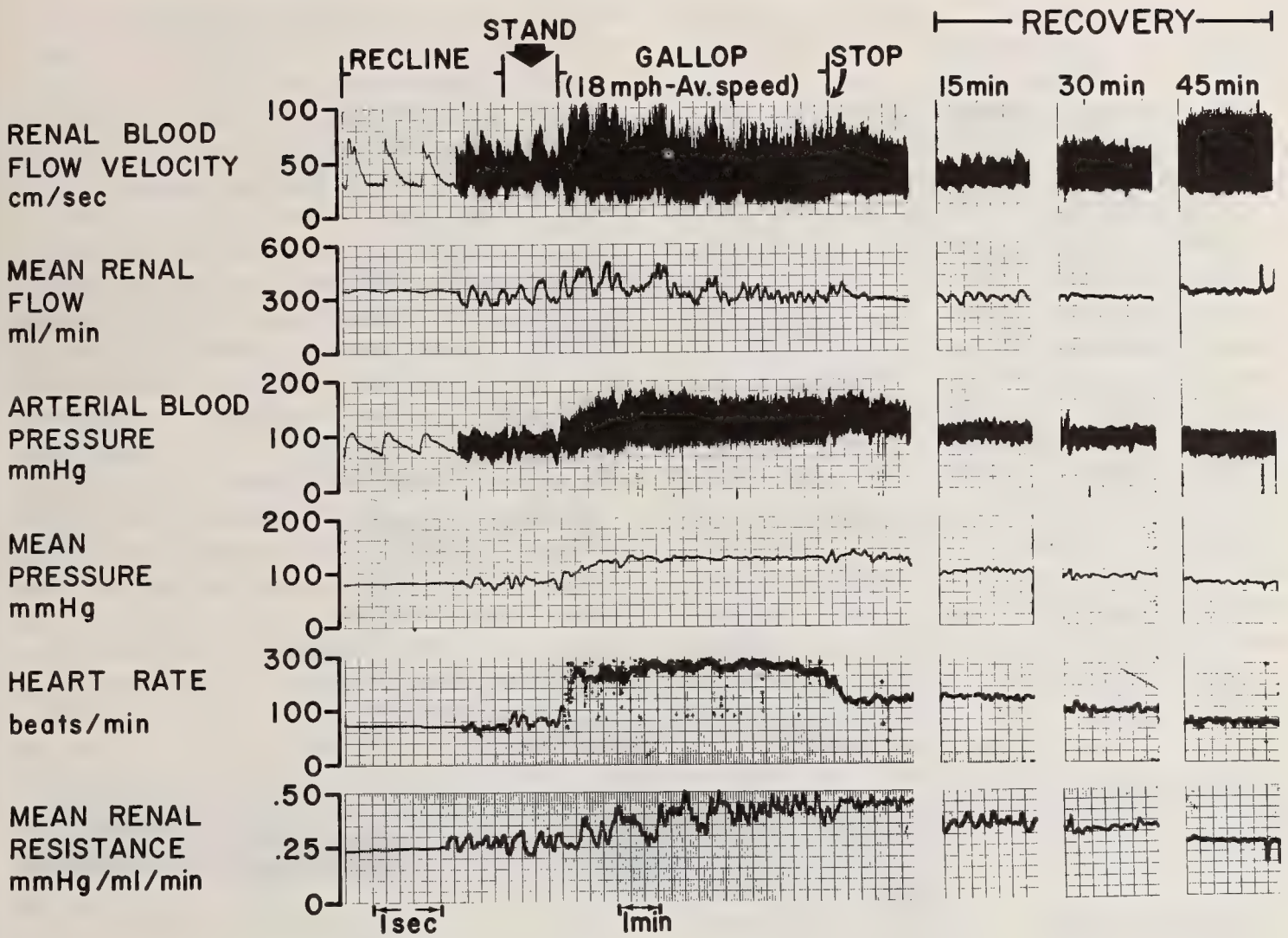


FIGURE 2.—A typical record of the renal response to severe exercise. From left to right the dog is reclining, then standing at rest, then running at an average speed of 18 mph, then resting during the recovery period. The phasic and mean values for renal blood flow and arterial pressure are shown along with computed heart rate and mean renal resistance. (Reprinted with permission from J. Clin. Invest. 50:1950, 1971).

series of dogs.[23] Oxygen-carrying capacity was limited by induction of chronic severe anemia (progressive phlebotomy and volume replacement) in a third series of dogs.[24] In each of these situations of circulatory impairment severe exercise produced much more intense visceral vasoconstriction than occurred in normal dogs resulting in sustained, marked reductions in visceral flows [7,23,24] (Figure 2). Thus, an important circulatory control mechanism was demonstrated only in the presence of circulatory impairment and could not be elicited in normal dogs.

Although the peripheral vascular response to exercise was clarified in dogs, the question remained why the canine response is different from the responses observed during exercise in man.[11–18] Studies in man utilizing indirect techniques to measure blood flows, e.g., PAH, indocyanine green, indicated that exercise reduces visceral blood flows and that the reduction in flow is roughly proportional to the severity of exercise. It is possible that the indirect techniques to measure regional flows in conscious man are not accurate indicators of total arterial inflow during exercise. For instance, it is possible that renal arteriovenous shunting occurs during exercise, which would make the PAH clearance technique liable to serious error, since that technique would only measure flow to the peritubular glomerular vascular network. On the other hand, it is possible that a species dif-

ference exists between man and the dog, resulting in a major difference in the peripheral circulatory adjustments to exercise.

One possibility, based on anatomical differences, is that the role of the spleen is significantly different in man and the dog during exercise, and that this organ represents a major circulatory reserve mechanism in the dog. It is possible that contraction of the canine spleen augments the circulating blood supply sufficiently to provide additional oxygen to the active muscular beds eliminating the necessity to divert additional flow from the inactive viscera. Accordingly, the peripheral vascular response to severe exercise was studied in another series of dogs before and after splenectomy.[25] Prior to splenectomy, exercise increased hematocrit from 40 to 48% while telemetered measurements of renal and mesenteric flow remained constant. Following splenectomy, exercise failed to increase hematocrit and compensatory reduction and diversion of visceral flow was observed. Thus, the spleen has an important role as a reserve mechanism in the canine response to exercise and this factor of species difference may explain the discrepancy between results of visceral flow during exercise reported from studies in man and in the dog.

To eliminate problems of species difference, it would be ideal to conduct experimental physiological studies in man. However, this is neither ethically possible nor are techniques currently available to directly measure cardiac dimensions, blood flows and pressures in man. The most attractive alternative is to directly measure these variables in a primate model of man.

To study circulatory dynamics in larger primates, telemetry is almost essential since these animals are not domesticated as are the commonly used laboratory animals, and have the capability of inflicting serious injury with their razorsharp teeth (Figure 3). Telemetry in this

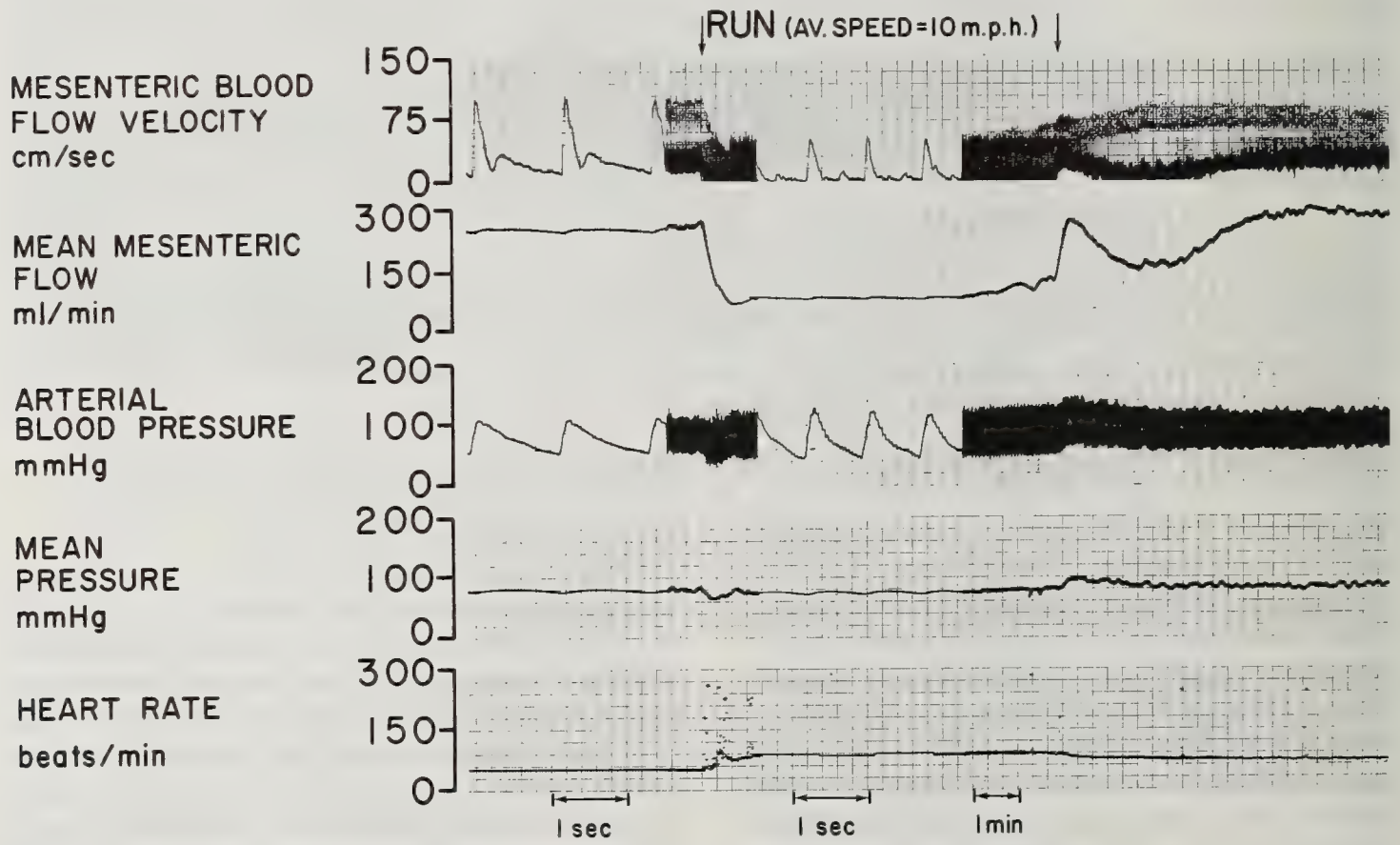


FIGURE 3.—A typical response of the mesenteric bed to severe exercise in the presence of circulatory impairment. This animal was in complete heart block. The phasic and mean values for mesenteric blood flow and arterial pressure are shown along with heart rate. The marked reduction in flow is best shown by the comparison of the phasic waveforms at rest (left) and during severe exercise (center). (Reprinted with permission from J. Clin. Invest. 50:1950, 1971).

situation enables the study of circulatory physiology in untethered, conscious baboons as they undergo a variety of spontaneous activities including eating, fighting, sleeping and postural changes (Figure 4). Studies to this date in conscious baboons have been primarily descriptive. For instance, we have studied the normal variations that occur in coronary dynamics in conscious baboons[26] and we have described the regional circulatory adjustments that occur in response to eating and digestion.[27] A natural extension of these studies, and those involving the response to severe exertion in dogs, will involve the study of the central and peripheral circulatory control mechanisms that are utilized in a variety of cardiovascular stressful situations in a primate model of man, e.g., the baboon.

SUMMARY

The system described in this paper can measure ventricular and arterial pressures, regional blood flows, and ventricular and aortic dimensions. Pressure is measured using a miniature pressure gauge chronically implanted within the ventricular chambers or within the lumen of the aorta. Dimensions are measured by recording the transit time of bursts of sound travelling between piezoelectric crystals sutured in opposition across the ventricle or aorta. Flows are measured using the ultrasonic Doppler shift blood flowmeter, i.e., measuring the Doppler shift in 10 MHz sound backscattered from the moving erythrocytes. Each of the electrical signals is converted to a frequency modulated subcarrier signal. These signals are impressed on a VHF FM carrier which is transmitted from the animal to the remote receiver and demodulators. The system electronics and power supplies are sufficiently small to be carried in a backpack by the animal, and sufficiently stable to allow measurements to be taken over periods of weeks.

Techniques for radiotelemetry of cardiovascular variables have been developed and applied to a variety of physiological studies. These techniques have enabled the study of animals living in habitat,[28] the elucidation of circulatory control mechanisms utilized during near maximal exercise and the use of larger primates as circulatory models of man.

REFERENCES

1. Patrick, T., Vatner, S. F., and Franklin, D. A system for radiotelemetry of left ventricular diameter and pressure in untethered animals. Proc. of the 24th ACEMB: 13:315, 1971.
2. Franklin, D., Vatner, S. F., Higgins, C. B., Patrick, T., Kemper, W. S., and Van Citters, R. L. Measurement and radiotelemetry of cardiovascular variables in conscious animals: Techniques. Proc. of Nat'l. Conf. in Res. Animals in Med., Washington, D.C. 1972.
3. Van Citters, R. L., and Franklin, D. L. Telemetry of blood pressure in free-ranging animals via an intravascular gauge. J. Appl. Physiol. 21:1633, 1966.
4. Franklin, D. L., Watson, N. W., Pierson, K. E., and Van Citters, R. L. A technique for radiotelemetry of blood flow velocity from unrestrained animals. Amer. J. Med. Electronics, 5:24, 1966.
5. Vatner, S. F., Franklin, D., and Van Citters, R. L. Simultaneous comparison and calibration of the Doppler and electromagnetic flowmeters. J. Appl. Physiol. 29:907, 1970.
6. Van Citters, R. L., and Franklin, D. Radio Telemetry techniques for study of cardiovascular dynamics in ambulatory primates. Ann. N.Y. Acad. Sci. 162:137, 1969.
7. Vatner, S. F., Higgins, C. B., White, S., Patrick, T., and Franklin, D. The peripheral vascular response to severe exercise in untethered dogs before and after complete heart block. J. Clin. Invest. 50:1950, 1971.
8. Rushmer, R. F. Constancy of stroke volume during exercise. Amer. J. Physiol. 196:745, 1959.
9. Rushmer, R. F., Smith, O., and Franklin, D. Mechanisms of cardiac control in exercise. Circ. Res. 7:602, 1959.
10. Vatner, S. F., Franklin, D., Higgins, C. B., Millard, R. W., Patrick, T., and Braunwald, E. The left ventricular response to severe exercise in untethered dogs. (Abstract) J. Clin. Invest. 51:99a, 1972.
11. Chapman, C. B., Henschel, A., Minckler, J., Forsgren, A., and Keys, A. The effect of exercise on renal plasma flow in normal male subects. J. Clin. Invest. 27:639, 1948.
12. White, H. L., and Rolf, D. Effects of exercise and of some other influences on the renal circulation in man. Amer. J. Physiol. 152:505, 1948.
13. Bucht, H., Ek, J., Eliasch, H., Holmgren, A., Josephson, B., and Werko, L. The effect of exercise in the recumbent position on the renal circulation and sodium excretion in normal individuals. Acta Physiol. Scand. 28:95, 1953.



FIGURE 4.—A baboon carrying instrumentation for measurement and telemetry of blood flow and pressure in a backpack. Since these animals are not docile, telemetry is essential.

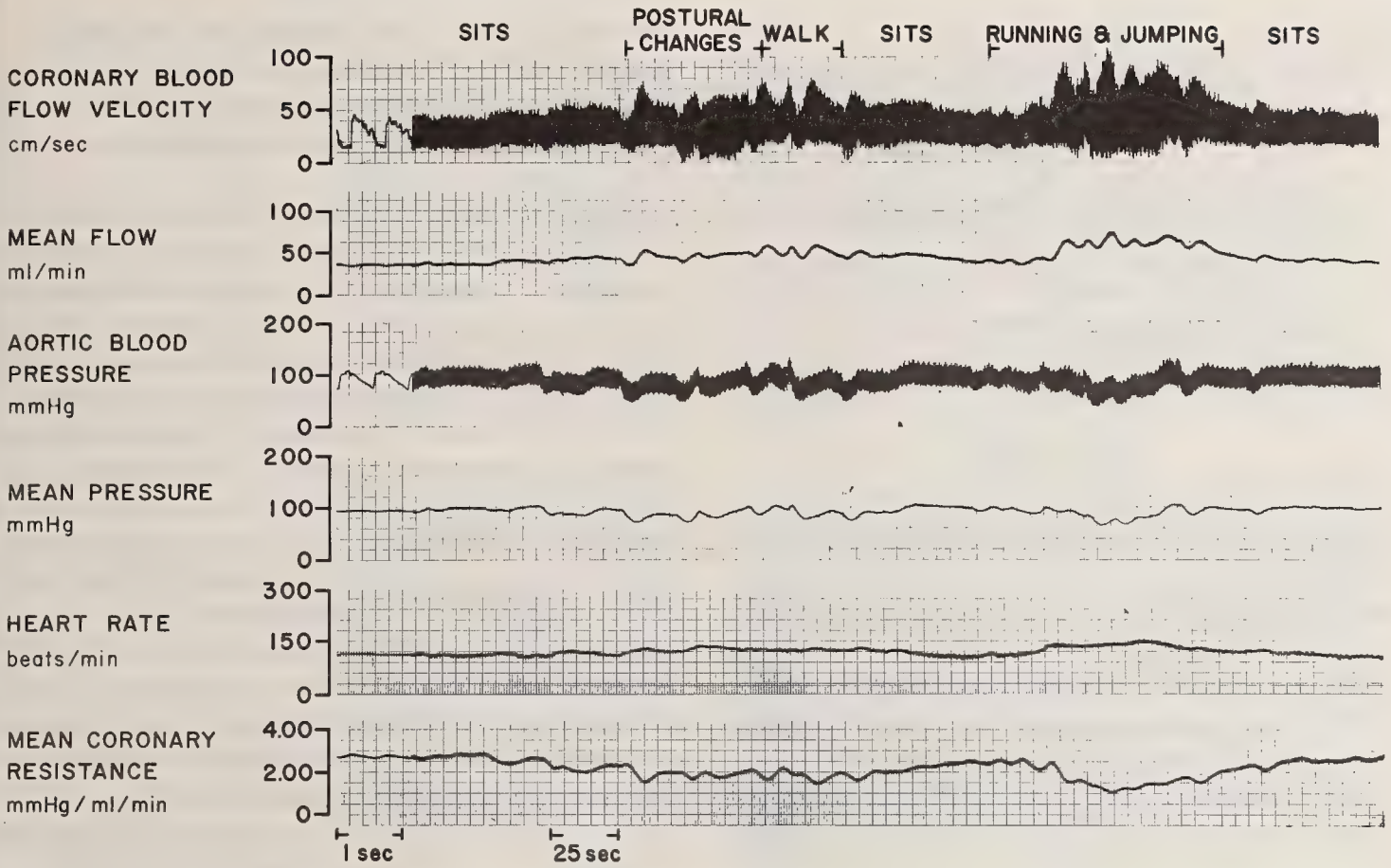


FIGURE 5.—A segment of recordings showing the response to a variety of spontaneous activities in a baboon such as the one shown in Figure 4. The phasic and mean values for coronary flow and arterial pressure are shown along with computed heart rate and resistance. (Reprinted with permission from Am. J. Physiol. 221:1396, 1971).

14. HARPUDER, K., LOWENTHAL, M., and BLATT, S. Peripheral and visceral vascular effects of exercise in the erect position. J. Appl. Physiol. 11:185, 1957.
15. WADE, O. L., COMBES, B., CHILDS, A. W., WHEELER, H. O., COURNAND, A., and BRADLEY, S. E. The effect of exercise on the splanchnic blood flow and splanchnic blood volume in normal man. Clin. Sci. 15:457, 1956.
16. ROWELL, L. B., BLACKMON, J. R., and BRUCE, R. A. Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. J. Clin Invest. 43:1677, 1964.
17. WADE, O. L., and BISHOP, J. M. The distribution of cardiac output in normal subjects during exercise. In *Cardiac Output and Regional Blood Flow*. Blackwell Scientific Publication, Oxford. 95. 1962.
18. BEST, C. H., and TAYLOR, N. B. The output of the heart and the regulation of its action. In *The Physiological Basis of Medical Practice*. The Williams & Wilkins Co., Baltimore. 302, 1961.
19. HERRICK, J. F., GRINDLAY, J. H., BALDES, E. J., and MANN, F. C. Effect of exercise on the blood flow in the superior mesenteric, renal and common iliac arteries. Amer. J. Physiol. 128:338, 1939.
20. RUSHMER, R. F., FRANKLIN, D. L., VAN CITTERS, R. L., and SMITH, O. A. Changes in peripheral blood flow distribution in healthy dogs. Circ. Res. 9:675, 1961.
21. VAN CITTERS, R. L., and FRANKLIN, D. L. Cardiovascular performance of Alaska sled dogs during exercise. Circ. Res. 24:33, 1969.
22. VATNER, S. F., HIGGINS, C. B., FRANKLIN, D., and BRAUNWALD, E. Role of tachycardia in mediating the coronary hemodynamic response to severe exercise. J. Appl. Physiol. 32: 380–385, 1972.
23. HIGGINS, C. B., VATNER, S. F., FRANKLIN, D., and BRAUNWALD, E. The effects of experimentally produced heart failure on the peripheral vascular response to exercise. Circ. Res. 31: 186–194, 1972.
24. VATNER, S. F., HIGGINS, C. B., and FRANKLIN, D. Regional circulatory adjustments to moderate and severe chronic anemia in conscious dogs at rest and during exercise. Circ. Res. 30: 731–740, 1972.
25. VATNER, S. F., HIGGINS, C. B., MILLARD, R. W., and FRANKLIN, D. Unpublished observations.

26. VATNER, S. F., FRANKLIN, D., HIGGINS, C. B., PATRICK, T., WHITE, S., and VAN CITTERS, R. L. Coronary dynamics in Unrestrained Conscious Baboons. Amer. J. Physiol. 221:1396, 1971.
27. VATNER, S. F., FRANKLIN, D., HIGGINS, C. B., PATRICK, T., and VAN CITTERS, R. L. Postprandial blood flow distribution in untethered baboons. (Abstract) Clin. Res. 20:402, 1972.
28. VAN CITTTERS, R. L., FRANKLIN, D., VATNER, S. F., PATRICK, T., and WARREN, J. V. Cerebral hemodynamics in the giraffe. Trans. of the Assoc. of Amer. Phys. 82:293, 1969.

DISCUSSION

CHAIRMAN FRANKLIN: So for those who didn't hear Dr. Scriabine's question, it was "How do you calibrate Konigsburg pressure gauges which are known to drift more-or-less over a period of time?"

A. SCRIABINE, Merck Institute, Pennsylvania: Calibration is one problem, but the second part is the drifting and the recalibration.

DR. VATNER: That's true. We do calibrate these gauges before implantation and on the bench. But as you know, these implantable gauges do have some degree of drift and so what we do in any of these experiments, if it involves more than a day, is to do a Statham calibration simultaneously. For instance, at the end of a day of exercise studies, we'll catheterize the peripheral vessel and do a simultaneous Statham calibration.

P. SOMANI, Abbott Laboratories, Chicago: You have very elegant studies showing normal and heart failure dogs. I wonder if your tests showed defect of digitalis glycoside in your model for heart failure and have seen it return to its normal values?

DR. VATNER: We have done digitalis studies in these heart failure dogs. We've mainly looked at the response of cardiac glycoside at rest. We've compared the peripheral vascular responses to ouabain in normal dogs in which digitalis causes increases in resistance in the renal and iliac beds and a decrease in resistance in the renal and iliac beds and a decrease in resistance in the mesanteric bed. For these dogs, which have had heart failure induced, ouabain then causes increases in blood flow and decreases in resistance to all beds. So there is a difference in response to cardiac glycoside. We haven't looked at the response to glycoside during exercise because we think that these dogs have mechanical lesions. This might be very difficult to overcome even with such a wonderful drug as digitalis.

C. G. LA FARGE, Children's Hospital, Boston: As always, the work of Dr. Franklin is perfectly beautiful. I'm very envious because I picked the wrong animal, in that I don't have the advantage of the field trips that you must be able to take. I'm sorely troubled, though, by that business of the end-diastolic fiber length. I know it's been reported from race drivers in Indianapolis, of sinus tachycardia at rates of 250, that they maintain a perfectly satisfactory output for obvious reasons, but I'm bothered by the fact that a dog exercising maximally at a heart rate of 300 is still not only able to increase his end-diastolic fiber length and volume, but as your slide also implied, increasing the stroke volumes as well. Are you sure that that 0.2 μsec method that you're using for measurement may not reflect geometric changes rather than an actual change in end-diastolic volume?

DR. VATNER: Well, I think just on a logical basis, if you want to, if you have some idea of the increases in cardiac output that must occur with this sort of exercise, the increases in heart rate that a dog normally gets from 80 to 300 involves a 2½ fold increase in cardiac output. But when you measure cardiac output, you know that even with treadmill exercises, cardiac output rises more than 2.5 times control, and it probably rises about 4 or 5 times control. This severe exercise, which is much more severe than the treadmill exercise, almost has to cause an increase in stroke volume as well. For this reason as well as the fact that if there is a misalignment of the two crystals, we'll probably lose our signal altogether, because these are very sensitive to alignment. If the alignment changes, then we will get drop-outs, mistriggerings, and we won't get useful data.

DR. LA FARGE: Have you calculated the actual stroke volumes in the two situations, rest and severe exercise?

DR. VATNER: No, we haven't done that as yet. We're just going to. We just completed this study and we're going over the data right now.

S. CASSIN, University of Florida, Gainesville: You had a measure of velocity on the organs that you were measuring flow in. You also measured or had a measurement for flow in one of the channels. It isn't quite clear to me how you go from velocity to flow without a measure of diameter? Were you measuring flow with another flowmeter, other than a Doppler?

DR. VATNER: No, we measure flow through the flowmeter and there are a number of ways in which you can convert to volume flow, as long as, in a chronic situation as these are, the vessel size within the transducer remains constrained. And we do this by assessing the internal vessel area, the external area, at surgery and the internal area at sacrifice of the animal. Also, we calibrate a certain percentage of these flow probes in vivo and vitro at the conclusion or at sacrifice of the animal. So in these situations, we have found the Doppler flowmeter to reliably indicate volume flow as well as velocity. In the acute situation, however, this may not be entirely true.

DR. CASSIN: You're assuming then, that the vessel size that you measure at the time of implantation is constant, because you have measurements of flow during the experiment and obviously you hadn't done the surgery at the end?

DR. VATNER: Yes. That's right. The assumption is during the experiment, the vessel size remains constant. What we do during these volume calibrations is alter pressure over a wide range, alter flow rates over a wide range and conduct calibrations at these levels. When it's found that the flowmeter still measures volume flow, no matter whether the pressure is altered or the flow rates are altered, then we can assume during the experiment, which was at a time very close to this, that it measured volume flow as well.

CHAIRMAN: An additional comment. Dr. Vatner also conducted comparisons with the electromagnetic flowmeter under these same conditions in a small number of cases and has found them to be identical. But I don't think we'll solve whether the Doppler flowmeter measures volume flow or not this afternoon. I think it does under these conditions.

QUESTIONER (unidentified): I had one other question concerning the decrease in renal blood flow that occurred under certain conditions. I presume that you had in mind that it decreased simply because of the decrease in oxygen supply to the tissues. Have you any data concerning the A–V differences or the oxygen consumption of the kidney under those conditions?

DR. VATNER: No.

QUESTIONER: Then you don't really know that in fact it does have a decrease in oxygen supply?

DR. VATNER: All we know is that cardiac output must have been severely limited during exercise.

QUESTIONER: Right. But the A–V differences are usually so small that I question whether or not there really is a decrease.

DR. VATNER: It's not the oxygen availability to the kidney that we were worried about. It's the oxygen available during exercise to the peripheral muscles and coronary beds that's important, and this is why the compensatory mechanism of diversion of viscera flow was utilized.

IMPLANT TELEMETRY AND STIMULATION

Wen H. Ko*

In some experimental procedures it is desirable to minimize disturbance of the animal under study by eliminating wires and other restraints, particularly for long term continuous experiments. In these cases, implant telemetry and stimulation can be considered. The added complexity, cost and possible technological difficulties should be justified by reduced interference with the subject. With advances in solid state electronic device technology, technical difficulties are being solved and implant electronic devices are expected to become important experimental instruments in the future.

INTRODUCTION

With the development of microelectronics and solid state device technology, electronic instruments can be made small and light enough to be implanted in the body of a living subject to perform measurements, stimulation, and control functions. The implant instrument is a valuable experimental technique for animal study and medical research and is becoming an acceptable device for diagnostic and therapeutic purposes. Advantages of implant instruments are: (1) minimum restriction or interference with the subject, and (2) long term operation without cross skin connection. The disadvantages include: (1) difficulty in designing small and light weight instruments, which raises the cost, (2) surgery and application skill, (3) the need to develop packaging material and long term power supplies, and (4) the lack of data on reliability and side effects of instruments and techniques being developed.

Implant instruments can be grouped into (1) telemetry for *in vivo* measurements; (2) stimulation, for generation of desired body response *in vivo;* (3) closed loop control systems; and (4) remote control manipulators. Telemetry and stimulation units are well developed for animal studies at the present. The implant control system and manipulators may be useful techniques for the future medical research. The use of implant instrumentation should be justified by weighing the benefits and cost to the experiment on an individual care basis. The major applications are as follows.

Animal study for physiology, pharmacology and disease

Implant instruments can provide nearly normal environment while the subject assumes regular activity. Continuous telemetering of body information can be obtained over a period of weeks, months, or even years. Desired body response can be controlled by electrical stimulation over a long period at a remote location. The implant heart pacemaker and blood pressure regulating device to control blood pressure are commercially available. Many other stimulators are being developed, including stimulators for bladder, brain, muscle, implantable visual aid for the blind and anesthesia machines, etc. These can be used for animal study as well as clinical applications.

Long term monitoring

Physiological parameters deep in the body of experimental subjects and patients may need to be monitored over a long period. For example, in the study of transplanted or artificial heart components, such as artery and valve sections, implant instruments may be used to monitor the flow, blood pressure, and other performance of these components.

Closed Loop Control System

An implant closed loop control system has many applications for biological investigation, such as the study of body regulations or substitution of a damaged control loop in the body

* Engineering Design Center, Case Western Reserve University, Cleveland, Ohio 44106.

to provide simple body functions or control of paralyzed limbs. For example, it is proposed to use processed control signals from ear or shoulder muscles, not used in normal activity, to stimulate and control the paralyzed limb muscle to recover some simple limb function.

Implant Manipulators

Implant manipulators can be used to release drugs or provide mechanical or electrical stimuli to perform therapeutic functions. For a certain type of cancer a nearly continuous release of a small amount of drugs into the blood stream over a period of months is desirable. The implant instrument will be a preferred method. Some of the spinal cord deformities or any other body malfunctions that require slow adjustment can also be performed by implant instruments when developed.

One can visualize many other implant instruments that can be used in artificial organs, prosthetic/orthotic devices, new therapeutic procedures, and medical research.

A literature survey[1-6] indicates that various telemetering devices have been developed and tested. These are implanted or surface mounted to transmit from 1 to 8 channels of signals from a living subject to a remote receiver a few inches to several hundred feet away. The subjects include humans, birds, fish, and other animals. The heart, bladder, muscle, peripheral and sensory nerves, brain, and spinal cord have been stimulated with electrodes from a single pair to about 100 units in parallel. Small motors and other electromechanical devices have been implanted to generate motion or displacement in the body and, in the laboratory, the one-dimensional motion of a limb has been controlled by EMG from the shoulder. However, the field of implant instrumentation is an "underdeveloped" area, with the exception of telemetering and, quite recently, the stimulation areas. They are technologically feasible, but before they can be realized many problems in materials, technology, physiology and psychology must be solved. This article discusses the state-of-the-art and problems of implant telemetry and stimulation. However, most of the discussion is applicable to the general field of implant instruments.

IMPLANT TELEMETRY

Implant telemetry is used to obtain information from within the body of an unrestrained living organism at a remote location through electronic transmission linkage. The block diagram of a typical telemetry system is shown in Figure 1. The implant transmitter unit is located totally within the body of the organism. The location may be intracavitary such as within the intestines, mouth, bladder, etc., or may be inside the body between the internal as well as external surfaces, such as subdermally or deep in the tissues. Surely, the same transmitter can also be mounted on the surface of the body when desired. The radio receiver and signal processor are remotely located to receive, record and display the information.

Design criteria of implant transmitters are:

(1) Small size and weight; less than a few percent of the size and weight of the subject;
(2) Minimum body reaction: packaged with nontoxic materials and with proper shape to reduce tissue reaction to the implant;
(3) High sensitivity (microvolts) and wide dynamic range, to handle signals ranging from microvolts to millivolts;
(4) Good fidelity;
(5) Low power consumption and long lifetime;
(6) Reliable, rigid, and easily handled;
(7) Suitable transmission range to permit the free movement of the subject and the use of units with the same frequency at nearby locations without interference;

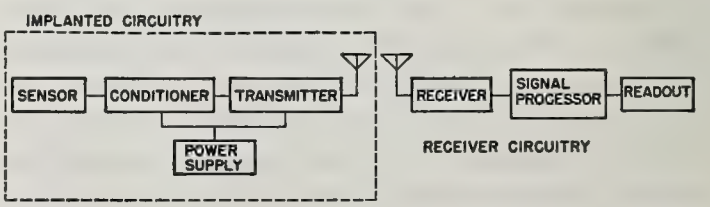


FIGURE 1.—Block diagram of a telemetry system.

(8) Compliance with Federal Communications Commission regulations for radio transmission.

Major considerations in selecting a telemetry system are:

(1) Characteristic of signal and performance required;
(2) Size, weight, and package as determined by the required life-span of the implant device;
(3) Implant location and techniques;
(4) Environmental interference and system requirements;
(5) Reliability and cost.

The earliest medical telemetry recorded was an FM link used to transmit pneumograms in 1948.[7] After the transistor became available about 1954, the telemetry development flourished. A good historical review of biomedical telemetry has been given by Caceres and Mackay.[4] Surveys and bibliographies on biotelemetry are also available.[3-6] Journals on biomedical engineering, medical instrumentation and telemetry usually carry recent developments in biotelemetry. Many physiological signals have been telemetered. The characteristics of these signals are summarized in Table I.

According to special considerations in each application, the engineering decision in designing an implant telemeter will include:

(1) Power Supply: Active (powered by battery or other active sources) or Passive (externally powered);
(2) Number of signal channels: single channel or multiplex;
(3) Range;
(4) Function: Tracking or signal transmission;
(5) Modulation scheme: (a) direct modulation or with subcarrier; (b) AM, FM, or PM.

Advantages and disadvantages of these choices are the same as for space and industrial telemetry and can be found in regular textbooks.

Active power supply may be chemical primary, secondary, nuclear batteries or energy converters which convert body energy into electrical energy. The passive implant telemeter may contain a resonant circuit in which the resonant frequency, made to vary with body signal, can be detected by a grid dip meter[8] or may contain transmitters powered by pulses of radio energy stored on a capacitor, or the transmitter may be powered by continuous radio energy supplied at other frequencies.[9]

Many single channel telemetry units have been used. Compelled by the limitations on size and weight, most of the present transmitting units use one or two transistors to obtain transducer, conditioner, and transmitter functions. With the new micropower techniques, these

TABLE I.—*Signal Characteristics of Telemetered Parameters*

Physiological Parameters	Transducer	Amplitude Range	Frequency Range
Electrocardiogram (EKG)	Electrodes	0.05—4 mV pulse	0.1—100 Hz
Electroencephalogram (EEG)	Electrodes	10—75 μV	0.5—200 Hz
Electrogastrograph	Surface electrodes	10—350 μV	0.05—0.2 Hz
Electromyogram (EMG)	Electrodes	0.1—4 mV pulse	2—10^5 Hz (10—500 Hz clinical)
Eye Potential; EOG or ERG	Electrodes	500 μV	0—250 Hz
Nerve Potentials	Electrodes	3 mV peak	up to 1000 pulses/second rise time 0.3 μsec
Bladder Pressure	Strain gauges	0 to 100 cm H_2O	0—10 Hz
Blood Flow	Electromagnetic flowmeter; ultrasonic flowmeter	1—300 cm/sec	1—20 Hz
Blood Pressure	Strain gauge on artery; hydraulic coupling to transducer; cuff gauges	0—400 mm Hg	0.5—100 Hz
Gastrointestinal Pressure	Variable inductance	20—100 cm h_2O	0—10 Hz
Intestinal Forces	Strain gauges	1—40 gm	0—1 Hz
Respiration Rate	Electrode impedance; piezoelectric devices; pneumograph		0.15—6 Hz
Stomach pH	Glass electrode; antimony electrode	3—13	0—1 cp-min
Temperature	Thermistor; thermal expansion	90°—100° F	0—0.1 Hz
Tidal Volume	Impedance; pneumograph	50—100 ml per breath	0.15—6 Hz

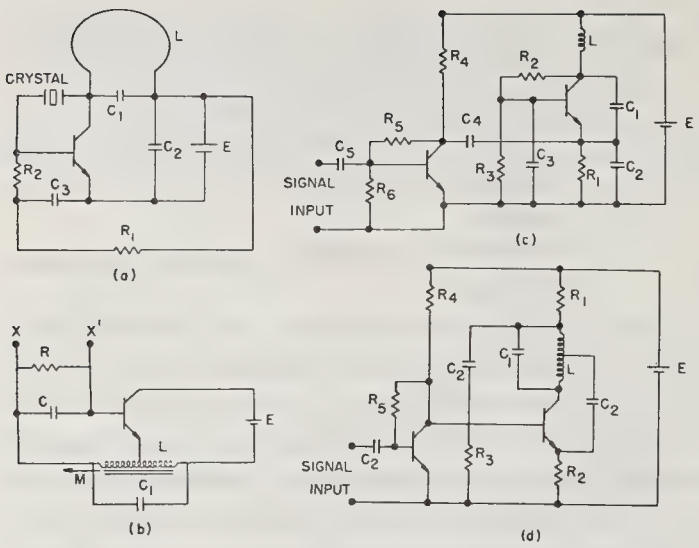


FIGURE 2.—Four popular telemetry transmitter circuits.

limitations may be removed. Figure 2 shows four popular transmitter circuits from the early literature.

The circuit in Figure 2(a) uses a piezoelectric crystal, as the feedback element in an oscillator, to achieve good carrier-frequency stability,[10] it is widely used for animal tracking and narrow-band signal transmission over a range from 30 meters to several kilometers; both CW and pulsed operation can be obtained by varying the value of resistor R_1.

The circuit in Figure 2(b) is a common-emitter Hartley-oscillator circuit;[11] the transmitter is modulated by varying the rate of pulses of radio frequency oscillations. It is popularly used to measure temperature, by replacement of the resistor R with a thermistor; pressure, by allowing pressure changes to move the core M; pH, by attaching suitable electrodes across the voltage input X; and other variables, by means of suitable transducers. Continuous wave operation can be obtained by reducing the value of R.

The remaining two circuits of Figure 2 are common-base oscillators; circuit 2(c) is a Colpitts circuit;[12] circuit 2(d) is a Hartley circuit.[13] Both use the voltage-sensitive emitter-to-base capacitance of the transistor to modulate the frequency of the carrier. These circuits, designed to transmit electrical signals such as electrocardiograms (ECG) and electromyograms (EMG), require relatively wide bandwidths of several kilohertz. The signal's amplitude may vary from 50 microvolts to several millivolts, and the transmission range is usually from a few meters up to 30 meters at a power consumption of milliwatts.

Figure 3 shows the two, single channel, continuous wave FM transmitters[5] developed at the Engineering Design Center of Case Western Reserve University. Table II lists the performance of the two transmitters. They can be powered by a #312 Mercury battery with an externally controlled magnetic reed switch to turn on and off the supply, or powered by RF induction power across the skin. For a 30″ x 24″ x 14″ cage, the RF power source required is 150 watts; for 18″ x 14″ x 14″ cage, 30 watts is required. The absorbed power density in the cage

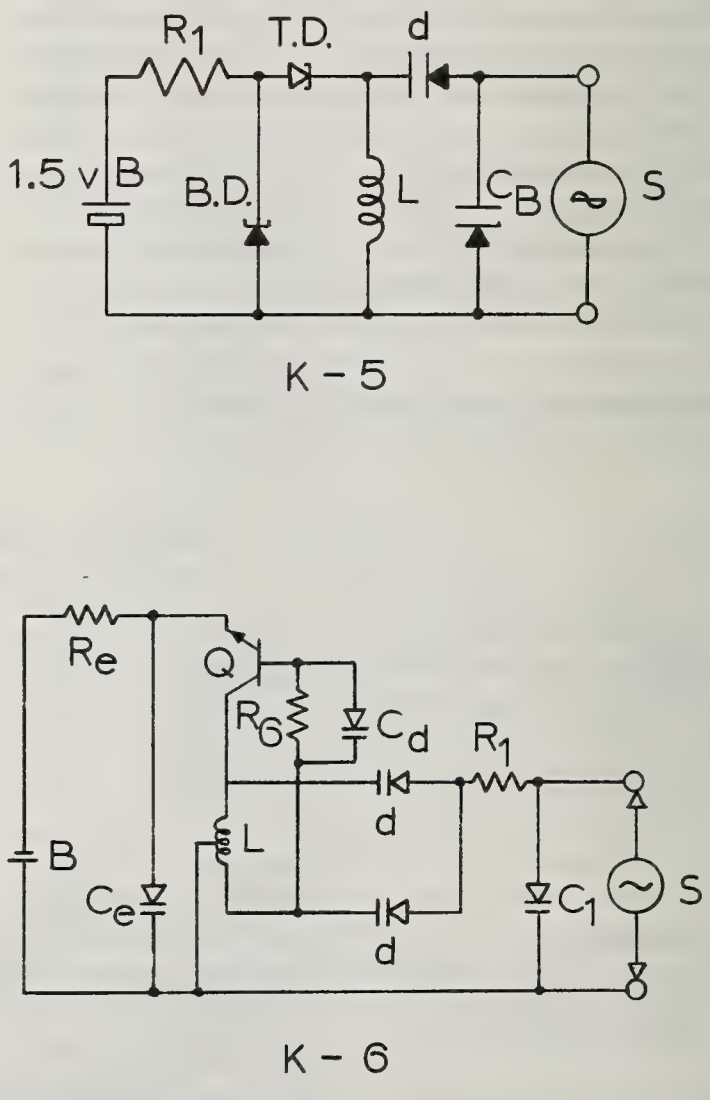


FIGURE 3.—Two single channel FM transmitters.

TABLE II.—*Performance of K–5 and K–6 Transmitters*

Item	K–5	K–6 Hybrid
Size, less battery	0.8 cm diam. × 0.2 cm	0.9 × 0.6 × 0.2 cm
Weight, less battery	0.44 gm	0.6 gm
Power Consumption	0.2 volt, 1.2 mA	1.3 volt, 0.5 mA
RF Frequency	100—250 MHz	100—250 MHz
System Noise (10 kΩ, 1 kHz)	3 μV	3 5 μV
Dynamic Range (limited by receiver	10^3	10^3
Frequency Response	0.1—20 kHz	0.1—20 kHz
Input Impedance	300 kΩ to several MΩ	300 kΩ to several MΩ
Transmission Range	5 μV at 1 to 2 m	5 μV at 1 to 2 m
Carrier Frequency Temperature Stability	Better than 0.05%°C	Better than 0.05%°C

is estimated to be between 10 to 20 mw/cm^2. The RF power received by a 1 cm radius spherical power detector is about 2 mW.

The K–5 and K–6 transmitters were implanted in mice, rats, rabbits, dogs and monkeys to transmit EMG and ECG with platinum electrodes. Packages with a replaceable battery, rechargeable battery, and RF power detector with or without reed switch were fabricated in lots of 50 to 100 units. Reliability data were obtained on these lots.

In order to reduce the overall size and weight, the power consumption of the transmitter should be minimized. With the new micropower transistors and design techniques, the power consumption can be greatly reduced. For example, the circuit shown in Figure 2(b) was redesigned[14] to provide a very low duty cycle of 10^{-4} to 10^{-5}. The M–3/K–6P single channel PFM transmitter consumes about 1 μW power and can be used for low frequency band signal such as temperature or pressure. A package using a #312 battery can operate continuously for over 6 months. The performance of the M–3/K–6P unit is given in Table III.

TABLE III.—*Performance of M–3/K–6P Unit*

Over-all size, including battery (M–3 plus K–6)	[20 × 12 × 5 mm] × 2
Weight of epoxy coated package (including #312 battery)	5 gm
RF frequency	120 MHz (others as specified)
Power supply voltage	1.3 or 2.6 volts
Battery current drain	0.5 50 μA
Operating temperature range	20°—45° C (others as specified)
On time of pulses	10 200 μsec
Duty cycle	10^{-4} 10^{-5}

A single channel PFM micropower transmitter with high input impedance amplifier was also designed.[15] The block diagram and performance of this transmitter are given in Figure 4 and Table IV, respectively. For long term monitoring, in order to improve the base line stability, a subcarrier is needed even for single channel transmission. This is similar to the multiple channel transmitters where subcarriers are used to separate the channels transmitting over the same RF frequency. For those frequency division multiplex systems, the subcarriers have different frequencies; the signal may use an AM or FM scheme to modulate the subcarrier. Both AM/FM and FM/FM multiplex systems with 4 to 8 channels[16,17] have been reported.

In the time division multiplex system, the signal channels are sampled and transmitted at different time slots. PAM/FM and PDM/PAM systems were designed at Case Western Reserve University[18,19] and the modular blocks were integrated into chips or flat packs. Depending on the signal frequency band, these systems cover 2 to 30 channels.

Receiving Systems

Due to restrictions on size and weight, implant telemetry transmitters are generally designed with minimum components required at low power level or operating in low duty cycle pulse mode. Therefore, noise and interference become serious problems at the receiving end. The transmitting frequency is generally not stabilized and drifts with time and motion of the subject due to change of loading. In order to maintain system reliability and performance, the receiver and demodulator should incorporate various noise discriminating schemes such as (a) Automatic Gain Control (AGC) for fading; (b) Automatic Frequency Control (AFC) and phase locked loop for frequency drift; and (c) noise limiter or amplitude discrimination for noise and interference.

For pulse modulated signal, the interference problem is especially serious. At Case Western Reserve University, pulse amplitude discrimination, pulse width discrimination and pulse frequency discrimination methods have been

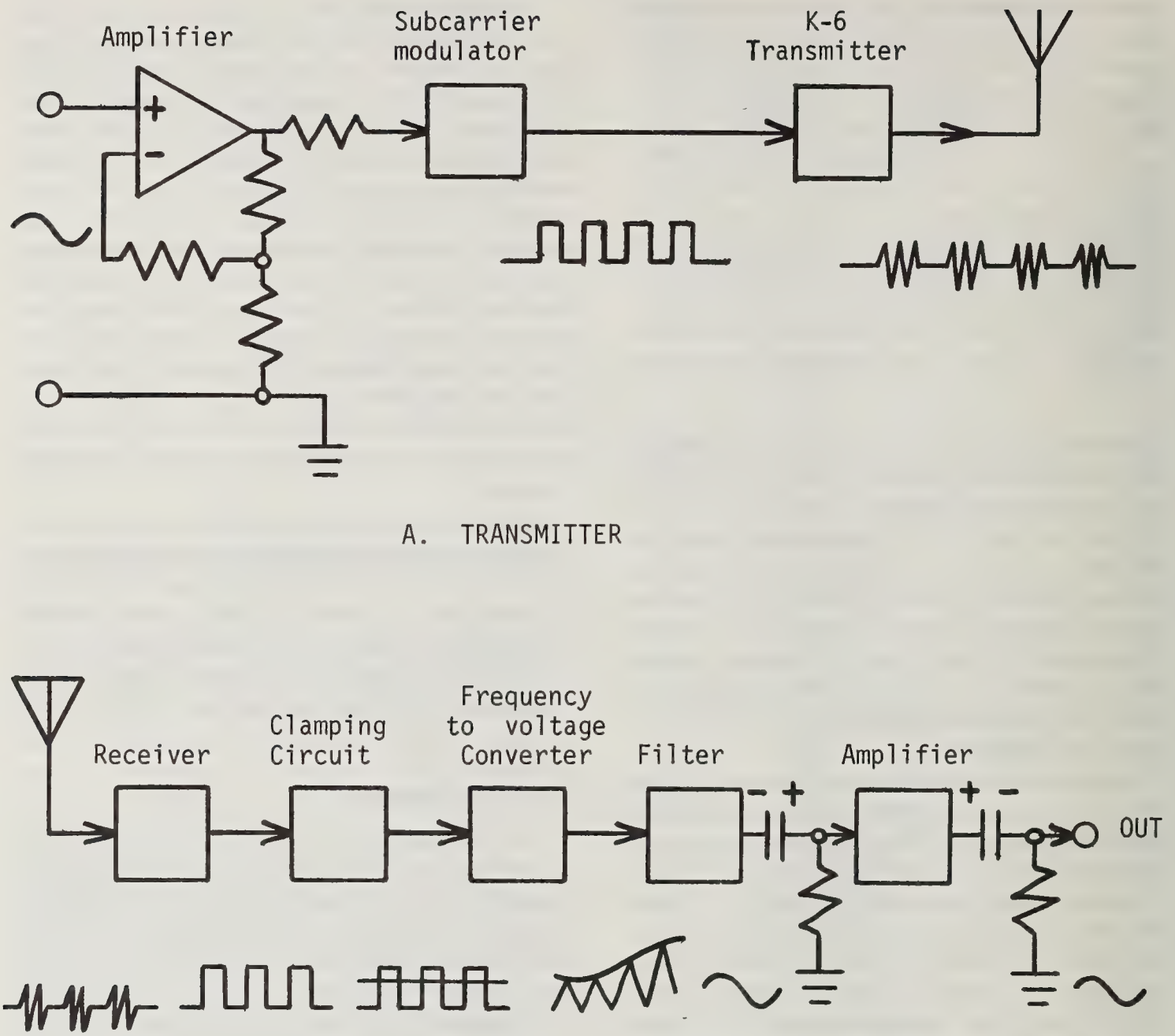


FIGURE 4.—Block diagram of single channel PFM transmitter.

examined together with the phase locked loop for pulsed signal to improve the system performance.

IMPLANT STIMULATION

Electrical stimulation of the nervous system for remote control of animals was tried as early as 1934.[20] However, its widespread research and clinical application is relatively recent. The heart pacemaker is the best known clinical device. In addition to the heart, stimulation has been applied to the bladder for voiding; the phrenic nerve for diaphragmatic contraction; baroreceptors for reduction of blood pressure; the spinal cord for pain suppression; and paralyzed muscle for regaining motor functions. With recent developments in brain stimula-

TABLE IV.—*Performance of PFM Telemetry Transmitter*

RF frequency	115 MHz
Signal bandwidth necessary for the receiver (I.F. stages)	1 MHz
Signal frequency band	0.4 500 Hz
Period for zero input signal	220 μsec
Modulation sensitivity	120 μsec/mV of input
Maximum input signal	±1 mV
Power consumption	100 μW at 2.6 volts

tion,[21-25] it is possible that it can be used to bypass sensory organs to generate hearing or some degree of vision directly at the central nervous system level as well as to temporarily alter personality or induce sleep.

The stimulating circuit is generally a simple radio-powered passive resonant circuit. Batteries, rechargeable batteries, radio power, and energy converters have been used to power these implant stimulators. An implant stimulator with feedback information is shown in Figure 5. The stimulating parameters for heart, bladder, nerves and muscles are summarized in Table V.

The major problems in stimulators are power supply, packaging, electrodes and tissue damage. The first two problems will be discussed later. Requirements for electrodes include:

TABLE V.—*Stimulating Parameters*

	Heart	Bladder	Nerve	Indirect Muscle (Via Nerve)	
				Intramuscular	Surface
Pulse width (msec)	0.1—10	0.1—10	0.05—1	0.1—10	0.1—10
Pulse Rate	30—200 ppm	15—35 pps	5—100 pps	1—50 pps	1—50 pps
Pulse voltage (volt)	0—9	0—10	0—10	0—10	10—60
Pulse Current (mA)	4—45	50	0.1—10	0.5—10	5—50

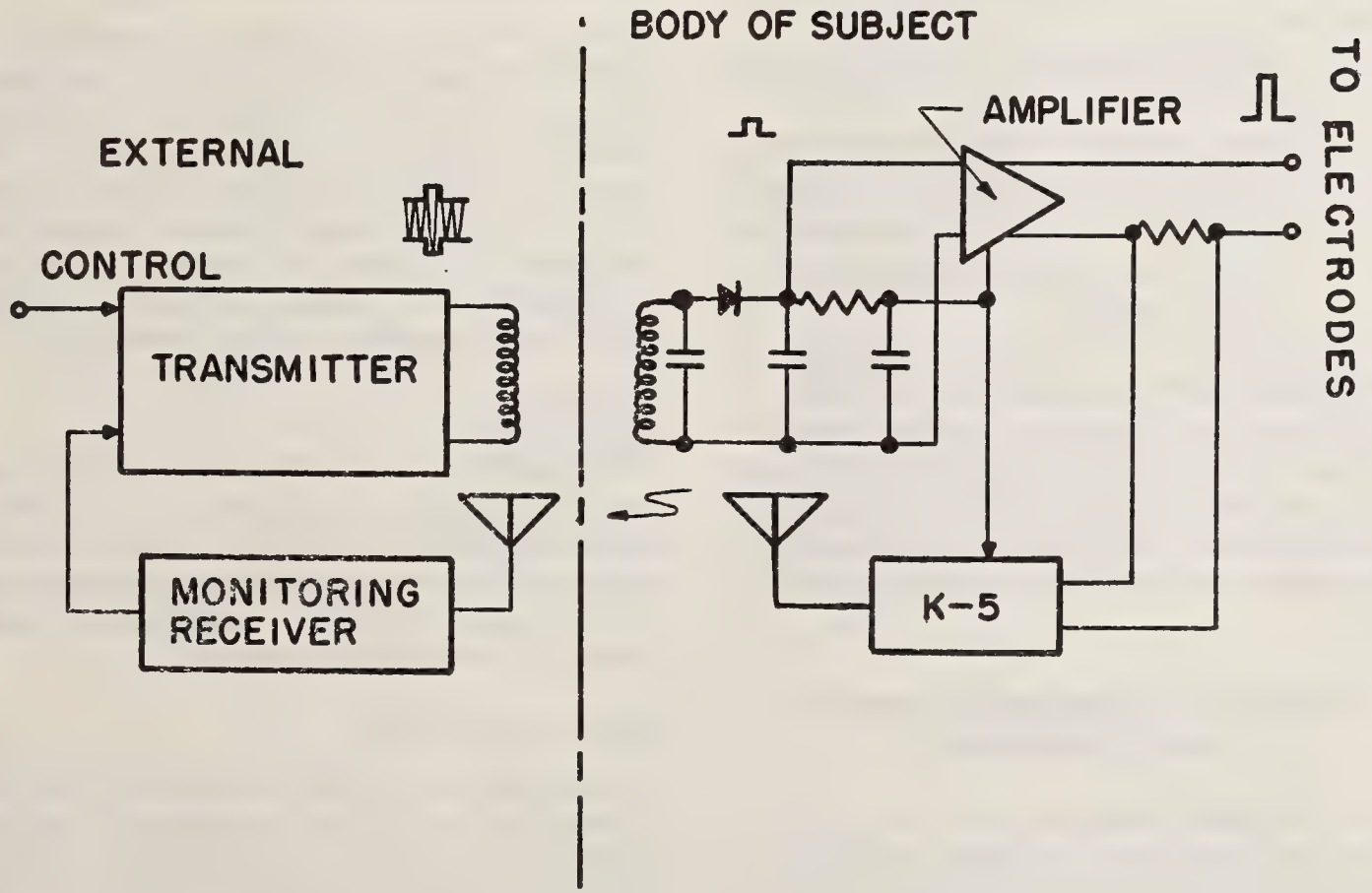


FIGURE 5.—Block diagram of implant stimulator circuit.

(1) Minimal corrosion and erosion by body fluid;
(2) Fatigue resistance to repeated stress; and
(3) Minimum body reaction to limit the increase of threshold due to peri-electrode fibrosis.

Multistranded wires of gold, platinum, stainless steel, and Elgiloy are used; however, coils or helical-wound multistranded wires provide better flexibility and less fatigue. Elgiloy is reported to have the best fatigue resistance, but corrodes seriously when used as an anode. Platinum electrodes have minimum corrosion and erosion when used for biphasic stimulation pulses. Bipolar electrodes are generally more effective than monopolar ones.

Many electrode problems remain to be solved. For brain stimulation, high density electrodes of the order of 1000 electrodes per cm^2 and the associated electronic circuits and systems are still to be developed.

Tissue damage resulting from electrical stimulation using "non-toxic electrodes" can be caused by:

(1) Gas generation due to large current density;
(2) Heat generated at the electrode site;
(3) Toxic material generated by electrode-chemical reaction at the electrode site;
(4) Mechanical stress caused by the electrode assembly.

These are subjects to be studied carefully together with the threshold of stimulation.

Although now in its infancy, stimulation is believed to have great potential. Many applications of stimulation to control pain, hormone secretion, mental disturbance, and to partially compensate for defective organs can be suggested.

PROBLEMS IN IMPLANT TELEMETRY AND STIMULATION

The following problems are the major research or development direction for designing implant instrumentation, telemetry and stimulation systems.

Circuit and System Design

Current implant instruments have relatively low reliability and poor stability compared to space or industrial electronics because of size and weight limitations. With micropower devices and integrated circuits (medium or large scale), more complex functions can be designed to consume less power and occupy much less space. Therefore, it is possible now to improve reliability, accuracy and stability of implant instruments within size and weight limitations.

Packaging Material and Techniques

Size and weight limitations also impose heavy requirements on packaging material and techniques, particularly for long term implants. Materials that can protect electronic circuits and power supply from harsh body environment for a period of a few years are still to be developed. Current materials including wax, epoxy and glass require too much weight and volume.

Body Reaction and Implant Techniques

Body reaction to implant instruments has not been thoroughly studied. Besides reaction at the implant site, total body reaction and possible long term side-effects should be studied. For example, (a) pressure exerted by the implant due to the weight has an effect on local circulation; and (b) besides the toxicity of foreign material, effects of surface conditions and shape of the implant package are to be studied.

Power Supply

A small, compact, lightweight power supply over a long period of time for implant instruments is another necessary component to be designed. The nuclear battery, solid electrolyte battery, and body energy converters present attractive directions of pursuit.

Implant Transducers

New design of implant transducers is needed to meet the overall requirements of size and weight. Current transducers, except electrodes, are not suitable for implant telemetry applications, and the design of transducers generally

involves many disciplines, requires higher technology and a longer time. Support in this area should be encouraged.

SUMMARY

This article has discussed the advantages, limitations and problems in implant telemetry and stimulation. Several micropower telemetry transmitters, along with passive and active stimulators suitable for implant in small animals were presented as examples. Transmitting and receiving schemes to reduce noise and interference were discussed with some pilot research. The problems of long term implant power supply and electronic packaging material as well as surgical techniques were outlined.

REFERENCES

1. Brown, J. H. U., Jacobs, J. E., and Stark, L. (Eds.). *Biomedical Engineering*. Davis, F. A., Philadelphia, Pa., 1971.
2. Clynes, M., and Milsun, J. (Eds.). *Biomedical Engineering Systems*. McGraw-Hill, New York, N.Y., 1970.
3. Mackay, R. S. *BioMedical Telemetry*. Wiley, J. New York, N.Y., 1970. 2nd Edition with reference lists on pages 455–525.
4. Caceres, C. A. (Ed.). *BioMedical Telemetry*. Academic Press, New York, N. Y., 1965.
5. Ko, W. H., and Neuman, M. R. Implant Biotelemetry and Microelectronics. Science 156, No. 3773:351–360, April 21, 1967.
6. Davis, J. F. Bibliography List on Biotelemetry. International Institute for Medical Electronics and Biol. Engineering, Paris, France, 1967.
7. Fuller, J. L., and Gordon, T. M. The Radio-inductograph—a device for recording physiological activity in unrestrained animals. Science 108:287, 1948.
8. Collins, C. C. Miniature passive pressure transensor for implanting in the eye. IEEE Trans. BME–14:74–83, April, 1967.
9. Ko, W. H., and Yon, E. T. RF induction power supply for implant circuits. Proc. 6th Intn. Conf. Med. Electr. and Biol. Eng., Tokyo, Japan, Aug., 1965.
10. Cochran, W. W., et al. Bioscience 15:98, 1965.
11. Mackay, R. S. Radio Telemetering from within the Human Body. IRE Trans. Bio-Med. Electron., 6(2):100, June, 1959.
12. Fischler, H., and Frei, E. H. Subminiature Apparatus for Radio-Telemetering of EEG Data. IEEE Trans. BME 10(1):29, January, 1963.
13. Young, I. J., and Naylor, W. S. Implanted Two-way Telemetry in Laboratory Animals. Am. J. Med. Electron. 3:28, January–March, 1964.
14. Ko, W. H., and Lin, W. C. A Study of Microwatt power pulsed carrier transmitter circuits. Med. Biol. Eng. 6:309. 1968.
15. Ko, W. H. Yon, E. T., Mabrouk, S. and Hynecek, J. Taped-on Heart Rate and Electrocardiogram Telemetry Transmitters. J. Am. Assn. Med. Inst. 5, No. 5, Sept.–Oct., 1971.
16. Robrock, R. B. II, and Ko, W. H. A six channel physiological telemetering system. IEEE Trans. BME–14:40–46, 1967.
17. Adey, W. R., et al. Design Considerations in Multichannel Telemetry. Proc. 19th Ann. Conf. Eng. Med. Biol. 1966.
18. Ramseth, D. J. An Optimal, Monolithic Integrated Circuit Multiple-Channel Biomedical Telemetry System. Ph.D. Thesis, Case Western Reserve University, Cleveland, Ohio. September, 1970.
19. Ko, W. H., and Hyneck, J. Micropower Pulse Modulated Multiplex Telemetry Systems. Proc. 9th Intn. Conf. on Med. and Biol. Engng., Melbourne, Australia, August, 1971.
20. Chaffee, F. E., and Light, R. U. Yale Journal of Biology in Medicine 17:83; 17:441, 1934.
21. Brindley, G. S. and Lewin, W. S. *J. Physiol. 196:* 479–493, 1968.
22. Delgado, J. M. R. Science 148:1361, 1965.
23. Richwien, R., and Millner, R. Med. Biol. Engrg. 4:193, 1966.
24. Shealy, C. N., Mortimer, J. T., and Reswick, J. B. A safe current for prolonged central nervous system stimulation. Proc. 20th Conf. Engrg. Med. and Biol., Boston, Massachusetts, 1967.
25. Silgalis, E. M. An Implantable Radio-Frequency Powered Electronic Stimulator. M.S. Thesis, Case Western Reserve University, Cleveland, Ohio. 1967.

CAPABILITIES OF IMPLANTABLE TRANSDUCERS

Eph Konigsberg*

ABSTRACT

The capabilities of implantable pressure transducers are set forth, both for standard specifications and for less commonly used criteria. Distinctions are made between expected performance in bench tests and *in vivo*, and between short term and long term expectations. The effect of the transducer on the animal and the implantation environment on the transducer are also set forth. Some estimates are made on probable expectations for long term implants.

INTRODUCTION

In this paper we primarily discuss implantable pressure transducers of the type which were first introduced some six years ago. These devices consist of a titanium disc 7.0 mm. in diameter, and 1.2 mm. thick, with a 2 mm. diameter stem, from which exits the transducer cable. Within this disc is an inner pressure reference cavity, behind the integral pressure sensing diaphragm. Affixed to the inner surface of the diaphragm are (usually) four semiconductor strain gages, arranged in a Wheatstone bridge. With proper excitation and compensation for temperature effects, an output signal is produced proportional to the pressure applied to the face of the disc. The discussion below will apply, with some modifications, to comparable instruments of smaller diameter or slight differences in thickness.

It is important to point out that we describe herein what can be expected on a 1 sigma probability. Transducers with improved or inferior capabilities may be obtained, either by selection, more careful process or manufacturing control, improved specifications, etc. Further, we describe herein, especially in our description of long term results, transducers which were designed and manufactured several years ago. Long term data regarding items now being produced must await the results of further tests now being conducted.

* Konigsberg Instruments, Inc., Pasadena, California.

METHODOLOGY

First, let us discuss the more standard specifications, and the results to be expected from a transducer which is now, and which is bench tested in the laboratory. The ranges given in the table below cover transducers which are from 3 to 7 mm. in diameter. Generally (though not always) the larger instruments will have the more preferable accuracy specifications:

Parameter	Units	Range
Non-Linearity	± % F.S., B.S.L.	0.2 to 0.5
Hysteresis	% F.S.	0.1 to 0.3
Non Repeatability	% F.S.	0.2 to 0.3
Static Error Band (Combined Non-Linearity, Hysteresis, and Non-Repeatability)	± % F.S., B.S.L.	0.3 to 0.6
Zero Temperature Shift	± mm. Hg./°C	0.1 to 0.3
Sensitivity Temperature Shift	± % reading/°C	0.2 to 0.3
Pressure Range, Std.	mm. Hg.	300
Output	mV/300 mm. Hg.	5 to 60

Some comments on the above: First, nonlinearity, hysteresis, and non-repeatability are generally such small numbers that they are almost always quoted in combined form. Second, those of you familiar with typical implantable transducer specifications will note that the errors or inaccuracies quoted above are small, in relation to the specifications usually quoted. This is for two reasons: we are discussing one sigma probabilities, and specifications have to take into account the occasional instruments which are slightly less accurate. Further, there is some degradation of certain aspects of performance *in vivo* and specifications attempt to take this into account.

There are additional accuracy and capabilities criteria which should be considered, and which

are less often quoted. In a bench test, the following performance would be expected:

Parameter	Units	Range
Frequency Response (Defined and Calculated) (undamped, in water, 1% flat)	KHz	1 to 4
Volumetric Compliance (Calculated)	$mm^3 \times 10^{-6}$/mm Hg	3 to 70
Flow Perturbation (at ¼ max. excitation)	Zero Shift, % F.S. Still/Agitated Water	0.2 to 2
Overpressure—Standard Arterial Transducer		
Less than 1% Zero Shift	mm. Hg	1000 to 3000
No Damage	mm. Hg	3000 to 9000
Gage Pressure Reference (to 10 Atm)		Yes
Line Pressure Error (Gage Pressure Reference)	% line pressure	0.1
Size—Diameter	mm.	3 to 7
—Thickness	mm.	1.0 to 1.2

We now consider the long term performance of transducers. The following tables reflect both long term laboratory tests, with transducers immersed in water and subject to simulated physiological pressure cycling, and *in vivo* experience.

Considering first the Standard accuracy and capabilities criteria, we have found the following figures to be representative:

Parameter	Units	Range
Non-Linearity	± % F.S., B.S.L.	0.2 to 0.4
Hysteresis	% F.S.	
Non-Repeatability	% F.S.	0.1 to 0.2
Static Error Band	± % F.S., B.S.L.	
Zero Temperature Shift	mm. Hg/°C	0.1 to 0.2
(Z shift induced)		±0.2 to 0.5
Sensitivity Temperature Shift	% reading/°C	0.4 to 1.0
(circuit induced)		0.3 to 0.4
Pressure Range—special		50 to ?

Several items are worthy of note. Nonlinearity, Hysteresis, Non-Repeatability, and their combination, Static Error Band, are almost inevitably improved with *in vivo* use. Indeed we have noted, as transducers come in for successive repair, that transducers continue to improve up to at least five years of age—figures beyond that are scattered, but similar. Second, the figures for zero temperature shift and sensitivity temperature shift are inevitably worse than bench test results, but do not represent a degradation of the inherent capabilities of the transducer.

As will be noted below, most transducers exhibit a long term zero shift (and to a lesser extent, sensitivity shift) *in vivo*. The temperature compensation network, being adjusted for the transducer's initial calibration, becomes less and less effective with time, because of this shift. If, however, a transducer is recompensated and recalibrated after prolonged use, its temperature characteristics will be comparable to, or *superior* to that of a new transducer; the exercising effect of use tends to make the transducer require less temperature compensation than a new instrument.

We now consider long term accuracy and capabilities criteria which are not commonly specified. The following tabulation represents my own judgment of the results of *in vitro* tests conducted in our laboratory and a preliminary report of *in vivo* tests conducted on comparable instruments by the Scripps Clinic and Research Foundation.

Parameters	Conditions	Units	Range	
Frequency Response	Probably only moderately diminished if no thick tissue deposition. No complaints.			
Zero Shift with time	Initial 1–3 months	% F.S./mo.	2	10
(in vitro tests)	Subsequent months	% F.S./mo.	1	3
Zero Shift with time	Initial 1–3 months	% F.S./mo.	2	10
(in vitro tests)	Subsequent months	% F.S./mo.	1	5
Predictability of Zero Shift with time	Any month	± % of previous month's shift	10	20
Sensitivity Shift with time—	Initial 1–3 months	%/mo.	1	4
(in vitro tests)	Subsequent months	%/mo.	0	2
Sensitivity Shirt with time—	Initial 1–3 months	%/mo.	3	10
in vivo tests	Subsequent months	%/mo.	1	6
Predictability of sensitivity shift with time	Any month (in vitro test)	± % of previous month's shift	±10	±20
Power Consumption	Std. 500 ohm	μA	50	300
(to generate a 1 mV signal F.S.)	Special 5000 ohm	μA	10	15
Resistance to Ground	Initial	M Ohms	100	∞
(Old style designs only)	3–6 months	M Ohms	50	>100
	6 mo—3 yrs.	M Ohms	20	>100

Several comments on the above tabulation are in order. The drifts noted above are not random in nature; except in rare cases, they tend to be unidirectional and hence predictable. Further, the researcher will usually reserve the more stable instruments for very long term or critical low pressure experiments. Indeed, the very unidirectionality of the drifts, demonstrating the systematic nature of the drift phenomenon, has led us to the redesign of the instruments to minmize this effect. Such tests are now in progress, and the short term (weeks) results are encouraging. Megohm leakage to ground has been measured on four instruments (5000 Ohm strain gages) which were in an animal for three years. The lowest value was 20 Megohms—which included the effect of a leaky cable. The sealed transducers, after being cleaned off and washed, had a low value of 100 Megohms, from strain gage to case; all units were operable. We believe newer cable designs of the last two years have overcome this weak point, as no comparable leaking has been reported in this shorter (two year) period of time.

We next consider the interaction of transducers and body. It is reasonable to state that leaking of transducers are virtually nil for the period of time they have been used. Transducers manufactured over five years ago have been sent in for repair, and are still functional. Some of these transducers have only been in the body intermittently. The analysis of the four transducers which had been implanted for three years corroborates the results observed on transducers sent in for repair.

There have been "Megohm" failures on both earlier and recent instruments. (We can consider an electrical leakage to ground as evidence of a moisture leakage to the interior of the transducer.) Recent failure analysis supports the view that such electrical leakage is the product of either poor design, improper assembly, or a combination of both, and should not be expected to occur.

Wire breakage and cable leakage should be virtually nil. We have only two years of experience with stainless steel cable, but in that period of time all failures have been attributable to improper installation. These almost invariably occur near the stem of the transducer, where ligatures have been tied around the cable, so as to secure it and position the transducer in the proper place in the left ventricle. Such ligatures can severely bend the wire which, upon repeated flexing, breaks. The addition of a reinforcement to the cable at that point, or greater care in tightening the ligatures around the cable will prevent this failure mode.

There has been some pin breakage with the transducer cable connectors in common use. Greater care, or the use of the new polarized connectors now available can minimize this relatively minor problem.

The influence of implantable transducers on the experimental animal are not well reported. We have not heard of great problems with clotting: the influence of surgical technique is pronounced. There are no systematic studies of the relative utility of coating transducers with heparinized colloidal graphite: both coated and uncoated transducers have been used with good results. The advantages of silicone versus PVC cables are equally unresolved. It has been reported that silicone seems to generate less rubor, but both types of cable have been used for long lengths of time.

The remaining principal problem, for systems which are not totally implanted, seems to be the design of the transducer cable connector. Where the usage pattern requires a long term experiment, where infection problems dictate that the end of the cable be buried under the skin, when the cable is first extracted from beneath the skin, used, disinfected (attemptedly) and then reburied, the design of the connector is such that infection often takes place, probably because there are reentrant crevices in the connector end of the cable.

SUMMARY

This paper has concerned itself with *present* performance of transducers (on bench test) and *past* performance of transducers on life test. On the basis of present laboratory results, we believe it to be realistic to predict that lifetimes of three years for both transducer and cable are currently attainable, and that drift rates (for both zero and sensitivity) considerably less than those quoted will be attainable within this year.

A redesigned transducer connector, easier to sterilize, should also be designed.

DISCUSSION

CHAIRMAN FRANKLIN: Thank you, Eph. Since you've implicated me in some of these data, I really must ask a question if I may. Let us pose a situation where I buy ten of your pressure gauges, ten of the P22's. I'm not concerned about the two that are almost perfect nor am I concerned about the six that are pretty darn good. How about those two that you put down on the bottom end? What kind of zero drift can I expect in the first two months from those two?

DR. KONIGSBERG: Now you know the answer to that because I saw it in your papers. You can get as high as 30 per cent drift per month, but that was one bad actor. I think between 20 per cent and 25 per cent drift would take care of the rotten two out of ten gauges.

CHAIRMAN: Well, I'd put a number some place in the middle there, but we're close to the same number in any event. Are there other questions for Dr. Konigsberg?

DR. KONIGSBERG: Oh, can I answer that just one more time? Just a second. I would say that we're looking at the prediction of this. One of the troubles that we've had (and I meant to say it, but I thought of it after I wrote the paper) was that we measured one batch of transducers and Dean Franklin measured another batch of transducers. It would be really meaningful to measure for a reasonably short period of time a batch of transducers and then take that same batch and then evaluate it *in vivo* because that is what he would like to see. He would be happy and most people would be happy to pay a premium and let us test them in our lab. Then take those bad 20 percenters, ship them to some place that doesn't know very much, or do something with them. Use them for chronic experiments at 50 per cent of list price and say it's only good for chronic. But the question is—can we correlate good versus bad in the laboratory, versus *in vivo*. I believe we can, and I think this is the next step, to buy a pre-selected transducer. Don't buy anything unless it's been in a cycling pressure system for a month, and this is being done now by NASA. We have a batch of ten or twelve transducers on life test. They're going to be measured every month and the ones that are going to be put in their biotelemetry systems will be the best ones. The worst ones, we'll keep looking at and they will either stabilize or we'll junk them. I think this is the approach.

We will know this in a year, because first we test them *in vitro* and then *in vivo*.

CHAIRMAN: But I'm not interested in what will happen in a year because I am buying ten pressure gauges and so on.

P. SOMANI, Abbott Laboratories, North Chicago: This seems very interesting for those who can afford to buy ten or twelve transducers. What about the poor guys who can buy only one, and happen to end up with this last 20 per cent?

DR. KONIGSBERG: You increase the price of a selected transducer by 20 per cent. This is what I was talking about. In short, what we're going to have to do is to use our pressure cycler, put an instrument in it, test it for a month. We will have to segregate those for stability, and, of course, we'll have to charge a premium price because we can't charge the same price and send it to some poor fellow who didn't know what he was buying. So what we will have to do then is select them and charge a premium price accordingly. We will give the worst transducers to people who have students and let them be used for student experiments or acute ones. But I think that the only way is to have us pretest them in a cyclic system for one month.

As I say, we are doing that now when we have the data correlated, you understand, with the data *in vivo*. Now, I suspect this correlation will be very good for reasons that would take too much time to go into, but I do think that will be fair. I think it would be welcomed by people purchasing one transducer.

D. B. JACKSON, Abbott Laboratories, North Chicago: Do you have any information or feedback about which telemetry system has had the most success with your transducers up to this point in time?

DR. KONIGSBERG: No, I do not. The ones I know of have been the units used by Franklin, some units that have been used with NASA

Ames, and some have been used with Sigmatron or Biosentry. They change, and there may be some home-grown ones. Those are the only telemetry systems I have any knowledge of and have received no comparative data at all. Indeed, the only long-term comparative data on drift that I've ever seen have been at Dean Franklin's lab. It costs a lot of money to reduce these data, and that's probably why most people either think it's great or not so great and don't put numbers down. So, until very recently, we haven't good numbers. We think it's 100 per cent a month or 50 per cent a month, but we haven't had numbers until very, very recently.

DR. SOMANI: May I ask a technical question? What happens when these transducers begin to improve with the chronic use, you know, how do they improve?

DR. KONIGSBERG: They improve because their linearity and hysteresis is invariably better. If you want to know specifically why they improve, the answer is that we noticed that we always require less balancing of the bridge. What happens is we take four string gauges that all match. They're all 500 ohms. After they are epoxied in place and closed up, we will find that they are 460, 480, and 470 ohms, for example. And because of this imbalance, we'll put in about 100 ohm or 120 ohm resistor to bring the bridge into balance. Invariably, well not invariably, but let's say 80 per cent of the time when we get a transducer back, we rebalance the bridge, because let's say a wire's been cut or something like that, we use a smaller value of resistance to balance the bridge. What is happening is that as a result of this exercising, the locked-in stresses from any epoxy that is cured tend to diminish with time. I might add that these locked-in stresses are not the cause of instability as we know it. We thought they were, but they are not. Surprisingly enough, it's other instabilities. But anyway, that's the reason. We always use less balancing and that's why we believe they're better. They're closer to their original state in the free condition.

K. SAGAWA, Johns Hopkins Medical School: I would like to ask about this chronic *in vivo* decalibration of zero point. How do people get the same reproducible zero reference point to check this *in vivo* transducer?

DR. KONIGSBERG: I cannot answer that question. You'll have to ask somebody who actually works with animals and he happens to be right here.

CHAIRMAN: Is Steve Vatner here? Would you care to answer that, Steve?

S. F. VATNER, University of California, San Diego: He'll have to repeat the question.

DR. SAGAWA: My question is how can you really test the zero point in a reproducible manner when you are concerned with 0.1 mm of mercury *in vivo*? I mean, using an extracorporeal string gauge.

DR. VATNER: Well, I think if you're concerned with .1 mm of mercury, that's out of the ball park that we're generally used to working in. If we can get within 4 or 5 mm of mercury, I think, that's as good as we can do with just a regular catheter implant and Stathum transducer measurement of pressure. So, maybe, we're interested in whether the mean pressure is 96 and if it's 98, it doesn't really matter. But if it is 96.5, you know, and we measure it 96, I don't think we're that accurate in any of our measurements.

DR. SAGAWA: The reason I raised this is again relates to Dr. Topham's thesis that venous pressure and interventricular pressure must be measured very accurately and that is really serious problem I think. To understand saturation, you just can't ignore venous pressure and end-diastolic pressure volume relationship.

CHAIRMAN: You can't ignore that it's an extremely important problem, but I also think that it's almost impossible to measure and that's why the earlier paper was, I thought, so very interesting. I think end-diastolic pressures and inferior venous pressures, in general, are an area probably of ignorance due to our inability to measure them. I think the point is very well taken. The instrumentation we have today, probably, is incapable of it, or turning it the other way around: an individual, who is drawing conclusions about the physiological status of end-diastolic pressure and its implications, better be very cautious in the interpretation of the output of his pressure gauge. Would you care to comment?

A. SCRIABINE, Merck Institute, West Point, Pa.: Are there more accurate transducers available?

DR. KONIGSBERG: Yes, they are. We have been making them on special order only so far. But we do have them available whether single or multi-channel. I do want to make one more comment about this 0.1 mm accuracy. If you will take these large numbers we have been throwing around, divide them by 30 days, and you can get something like a millimeter of drift a day with one of the worst systems down to 0.5 or 0.1 mm drift a day.

In the measurement of cerebral spinal fluid pressures in the brain, where the transducers are put in and calibrated over a three to four day period, it is important to measure very low pressures. It has been commonly reported that stability on the order of a millimeter of mercury a day is achievable now. So it all depends on whether you want to know two months from now your pressure to within a millimeter of mercury or you can calibrate immediately before and after an experiment because a dramatic change is not going to occur in a day. Thus, if you are making a study and you can calibrate or get a few calibrations, you will get some kind of a line connecting your points. You can have some reasonable assurance that you are accurate to within a few millimeters of mercury, if you can stop the experiment, sacrifice the animal, and calibrate your transducer. You will not be positive about what happened several hours or days ago, but you are going to be reasonably close. Now, this does require technique; it is expensive; it does require care. But the thing that is most important is, except for a few bad actors, that these random shifts do not occur. They are, in general, systematic shifts.

A MATHEMATICAL MODEL FOR MEASURING BLOOD FLOW BY RESIDUE DETECTION WHEN RADIOTRACER RECIRCULATION INTERFERES

K. B. Larson and D. L. Snyder*

We have developed a mathematical model for measuring blood flow in a vascular system by external monitoring of radiotracers when tracer recirculation is not a late event and must be taken into consideration. Additionally, the model accounts for interfering recirculation of tracer to other perfused tissues also within the detector field of view. Central to the model is the use of two injections of tracers—an intra-arterial and an intravenous—upstream and downstream of the particular organ or region of interest. Thus, two residue curves are obtained. We have developed equations indicating how to employ the two residue curves in order to determine the mean transit time of tracer through the vascular system of interest, as well as higher moments of the transit-time distribution if these are desired. The requisite numerical calculations need not rely on curve-fitting of the data. Although our method can yield compartmental parameters if these are appropriate and desired, our equations do not depend for their validity on any model of tracer transport within the system of interest. We describe some experiments designed on the basis of our dual-injection method to account for recirculating tracer in blood-flow measurements of various organs of anesthetized dogs, and give some indication of the agreement between blood-flow values predicted on our theory with those determined independently.

INTRODUCTION**

Zierler[1] was the first to show, without invoking special assumptions as to transport mechanisms in a vascular region of interest, that the ratio of blood flow to volume of distribution of a radioactively-labeled indicator could be measured by *external* monitoring of the radioactivity following rapid arterial injection of the indicator. He has called this technique *residue* detection, contrasting it with *outflow* detection.[2] The latter is synonomous with the term "indicator dilution," in which the flow-weighted *concentration* history of tracer in the venous blood is continuously monitored by use of a catheter or by other means.

The mathematical analyses central to the interpretation of kinetic tracer data in most of the circulation studies reported in the literature have been based on models which, in the interest of simplicity, have ignored recirculation. Such models have been widely employed both in residue and in outflow detection. An important limitation of these models is that they can be used successfully only in those situations in which the major portion of the response curves are obtainable prior to onset of the first recirculation of tracer. A further complication which limits the usefulness of the models used in residue detection is due to interference from radiotracer recirculating into perfused regions adjacent to the system of interest and within the field of view of the detector.

When recirculation is a late event, an arbitrary extrapolation of the primary response curve does not result in large error.[3] However, when recirculation appears relatively early after injection of tracer, the primary response curve cannot be recovered confidently in this simple fashion. In such a circumstance, a principle first suggested by Stephenson[4] can be applied. According to this principle, injections of tracer and concentration measurements are required at multiple sites in the cardiovascular system. This permits specific account of recirculation to be taken *ab initio* in the mathematical model, and allows the transit-time distribution for the vascular system of interest to

* School of Medicine, Washington University, Saint Louis, Missouri.

** The theoretical studies reported herein were supported by National Institutes of Health Research Grant RR-00396 from the Division of Research Resources.

be recovered by means of a deconvolution procedure.

Because of certain relative advantages offered in some cases by the use of residue detection, it appears useful to extend its application, via Stephenson's method, to measurements in which early recirculation interferes with the primary response. The purpose of this paper, therefore, is to present theoretical results which allow measurement of blood flow by residue detection when recirculation cannot be ignored. In it, we discuss the mathematical analysis by means of which mean transit time and higher moments of the transit-time distribution of blood flow in a vascular bed can be deduced from externally-monitored radioactive tracer being transported through the bed by first and subsequent circulations. We show that in certain favorable cases, our method is capable of accounting for interference due to recirculating radiotracer carried into perfused regions within the field of view of the detector and adjacent to the vascular bed of interest. No appeal to any transport model is made in our analysis. The results are therefore of general validity in the sense that they are independent of any special assumptions as to the transport mechanisms involved. In particular, we do not invoke the requirement of fully dispersed flow basic to compartmental analysis, nor do we make any assumptions as to the degree of equilibration of tracer between vascular and extravascular spaces. Because it does not depend on transport models, our method implies nothing about the shapes of experimental response curves. Consequently, the computational procedures for deducing the mean transit-time and higher moments of the transit-time distribution from experimental data can be exclusively numerical if so desired.

MATHEMATICAL MODEL

For the present application, the circulation is properly modeled as a closed-loop flow system. Figure 1 is a schematic representation of the cardiovascular system in its essentials. The myocardial circulation is considered as encompassed within the system labeled systemic circulation, while the components labeled right heart and left heart are considered to represent flow systems consisting of the blood within the heart chambers. All blood vessels are regarded as being components of either the systemic or the pulmonary circulations, so that the arrows leading between components may be considered merely as delayless and dispersionless transfer lines.

In the residue-detection method of measuring blood flow,[2] a bolus of radioactively-labeled tracer is injected into the inflow of the system of interest, and the subsequent content of tracer in the system is monitored as a function of time by means of a suitable radiation detector collimated over a region containing the system. In many cases, recirculating radioisotope interferes with the primary clearance record which would be obtained in the absence of recirculation. This interference occurs in two ways, viz., (a) by contributing added radioactivity to the system of interest, and (b) by contributing added radioactivity to other systems adjacent to the system of interest which are also within the field of view of the detector. We have found that the problem of obtaining the transit-time distribution and its moments for a particular system by means of residue detection in these circumstances can be solved through application of Stephenson's principle. For this purpose, we employ, in addition to an arterial injection upstream of the system of interest, a second injec-

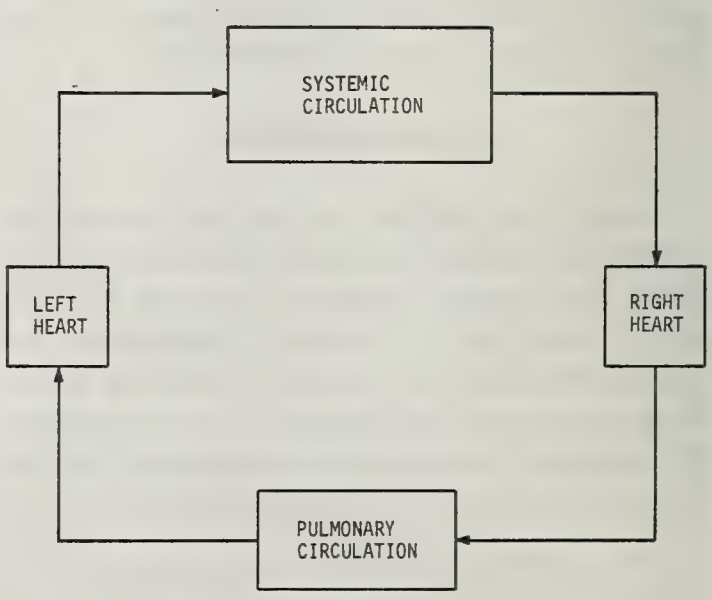


FIGURE 1.—Schematic Representation of the Mammalian Circulation.

tion of radioactive tracer at the venous outflow. We show our method to be valid when there is recirculation of tracer to the system of interest whether or not adjacent tissues are also within the detector field.

Figure 2 represents the flow model in more detail than Figure 1. Let the interior, D, of the circle in this figure represent the field of view of a radiation detector used in a residue-detection measurement. An arbitrary portion of either the systemic or the pulmonary circulations is contained within D. Flow elements, which may be blood vessels, vascular labyrinths, vasculatures of entire organs, or the entire systemic or pulmonary circulations, are characterized by their system functions $h(t)$, $G(t)$, and $F(t)$. These functions are the transit-time frequencies,[4,5] which characterize the various flow elements. Let $h(t)$ represent the system whose flow parameters we wish to determine, and let an arbitrary number of systems $G(t)$ be also present within D. Let $h(t)$ and the $G(t)$ be interconnected to form a flow system of arbitrary complexity within D. Let the composite system consisting of all systems outside D have a resultant transit-time distribution $F(t)$. The component subsystems in $F(t)$ can be arbitrary and can intercommunicate in an arbitrary fashion. Recirculating tracer can enter and leave the region within D and the system $F(t)$ by means of an arbitrary number of inlets and outlets through which the fluxes $x(t)$ and $y(t)$ can be convective or diffusive. Let the rates at which tracer is metabolized, sequestered or otherwise eliminated from the circulation be given by $z(t)$. Directions of tracer flux between elements in Figure 2 are indicated by arrows. The interconnecting lines can be thought of as delayless and dispersionless transfer paths, with the double lines representing multiple routes. The single lines associated with $h(t)$ refer to the fact that we are concerned here with describing a method of measuring flow only in systems having a single convective inlet and a single convective outlet. Diffusion of tracer across the boundaries of the system of interest is inadmissible if the mean transit time obtained from the measurements is to be used for determining blood flow. (Systems with multiple convective inlets and outlets may be treated by the present method through the use of more than two injections; however, we shall not pursue this question here.)

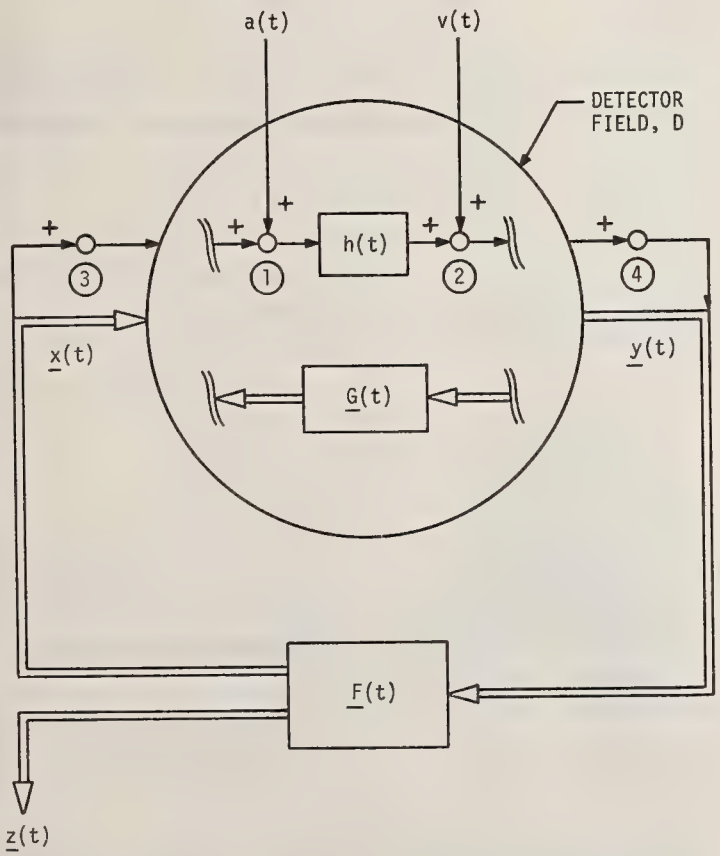


FIGURE 2.—General Model for Residue Detection.

The usual requirements for the validity of tracer stimulus-response methods concerning linearity and stationarity are assumed to hold for the systems we consider here. Thus, the elementary and composite systems depicted in Figure 2 are assumed to be linear only with respect to tracer stimuli and responses, a condition easily met.[6] The systems are not required to be linear with respect to changes in concentration nor with respect to perturbations in flow rates of traced substance. Instead, the system is regarded as being in a steady state, that is, concentrations and flow rates of traced substance are assumed to be time-invariant. It is further assumed that $h(t)$ for the tracer is the same as that for the substance being traced. This implies that no changes in chemical properties of the label which would affect its kinetic behavior within the detector field are permitted. Thus, if the label undergoes metabolic changes anywhere outside the detector field D, none of the products of metabolism incorporating radioiso-

tope may be permitted to enter D by recirculation; instead, they must be sequestered or eliminated.

The radiotracer stimuli are injected at rates $a(t)$ and $v(t)$ at the arterial and venous sites, respectively. The injection experiments are described mathematically as follows:

$$\text{Injection experiment } A \quad \begin{cases} a(t) = i_a(t)\, u(t), \\ v(t) = 0, \end{cases} \tag{1}$$

$$\text{Injection experiment } V \quad \begin{cases} a(t) = 0, \\ v(t) = i_v(t)\, u(t). \end{cases} \tag{2}$$

Here, $u(t)$ is the unit positive step function defined by

$$u(t) = \begin{cases} 1, & t \geq 0, \\ 0, & t < 0. \end{cases}$$

We thus identify $t = 0$ as the start of injection for each experiment. The injection rate functions $i_a(t)$ and $i_v(t)$ may have any forms provided only that the integrals

$$\mu_n = \int_0^\infty t^n i_a(t)\, dt$$

and

$$\nu_n = \int_0^\infty t^n i_v(t)\, dt, \quad n = 0,1,2, \ldots \tag{3}$$

defining their nth moments exist. In particular, μ_0 and ν_0 must represent, respectively, the total amounts of tracer q_{0a} and q_{0v} administered in the two injections. The requirements for the location of the two injection sites are: (a) that the entire upstream or arterial bolus, on first transit of the detector field D after injection, pass through $h(t)$; and (b) that the entire downstream or venous bolus, on first transit of D after injection, follow the same route(s) as departing tracer from the first-transit arterial bolus. Stated differently, between the two injection sites, only the system of interest may intervene. A further equivalent interpretation is that the present method yields the transit-time distribution of the composite system lying between any two injection sites satisfying conditions (a) and (b) above. Thus, for real vascular systems in which injections of tracer must be made into blood vessels at locations somewhat removed from the vascular bed of interest, the measured transit-time distributions will reflect additional delay and dispersion of the boluses due to their passage through these vessels. In view of the foregoing discussion, sites 1 and 2 in Figure 2 are seen to be appropriate locations for the arterial and venous injections, while sites 3 and 4 are not.

The responses corresponding to the stimuli described by equations (1) and (2) are denoted by $q_a(t)$ and $q_v(t)$, respectively. These functions represent the amount of radiotracer present within the detector field at any time after the beginning of injection experiments A and V, respectively, in excess of any tracer already present in D from previous injections. The detector responses observed will be proportional to $q_a(t)$ and $q_v(t)$ provided the effective detection coefficient remains time-invariant. Some response curves similar in form to those which might be obtained from the two injection experiments indicated in Figure 2 are shown in Figures 3 and 4, where the normalized responses

$$\tilde{q}_a(t) = q_a(t)/q_{oa} \tag{4a}$$

and

$$\tilde{q}_v(t) = q_v(t)/q_{ov} \tag{4b}$$

are plotted. The hypothetical response curves in

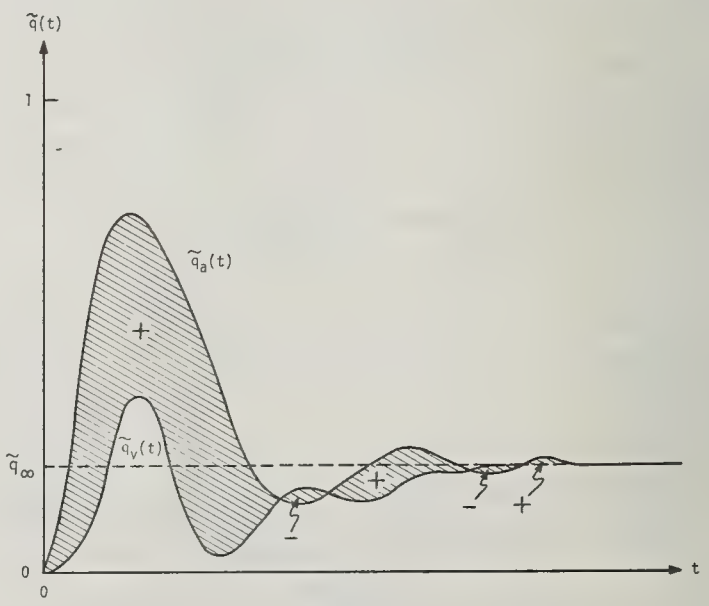


FIGURE 3.—Hypothetical Arterial (a) and Venous (v) Detector Responses for Model of Figure 2 without Elimination of Tracer.

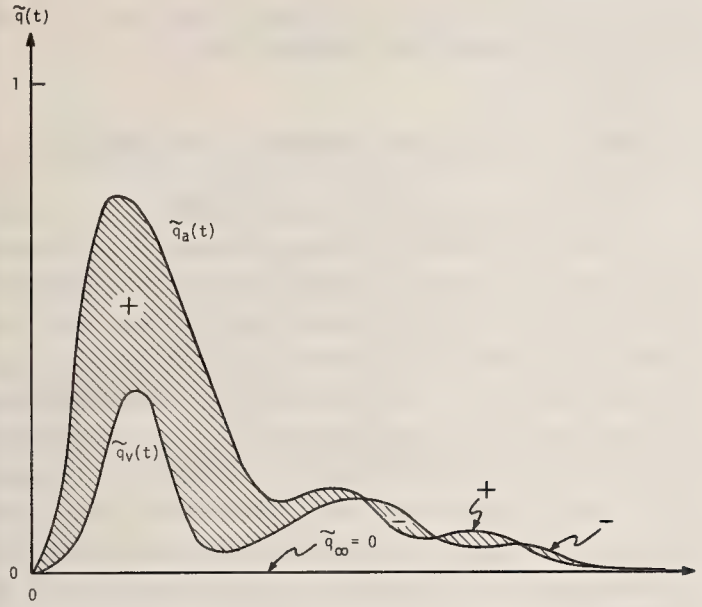


FIGURE 4.—Hypothetical Arterial (a) and Venous (v) Detector Responses for Model of Figure 2 with Elimination of Tracer.

each of the figures are shown exhibiting multiple recirculation peaks, and each curve is shown approaching, with increasing time, a common normalized steady-state value, defined by

$$q_\infty = q_a(\infty) = q_v(\infty). \quad (5)$$

Here $q_a(\infty)$ and $q_v(\infty)$ are the normalized steady-state values of the amount of tracer within D for the two injection experiments. These can be nonzero only when there is no loss of tracer from the system during the course of the measurements. In this case, the tracer elimination or sequestration rate is zero. When this rate is positive, the final steady-state values $q_a(\infty)$ and $q_v(\infty)$ will be zero. This is the situation depicted in Figure 4.

MEAN TRANSIT TIME

A detailed analysis of the model described above appears elsewhere.[7] We provide here the principal results which we have deduced from the model by applying the principle of conservation of tracer mass together with elementary linear systems theory.

The first moment

$$t = \int_0^\infty t h(t)\,dt \quad (6)$$

of the transit-time distribution, or mean transit time,[5] is given on our model by

$$t = (1 - q_\infty)^{-1} \int_0^\infty [q_a(t) - q_v(t)]\,dt. \quad (7)$$

(Our model is also capable of yielding higher moments of $h(t)$. In Reference 7 we give explicit expressions for these. We show further that by using the higher moments it is possible to compute the parameters of a model conjectured to represent the transport processes for the region of interest. The particular representation selected in Reference 7 to demonstrate such a determination is the multicompartmental model.)

The above result allows the mean transit time to be obtained from the experimental responses by purely numerical methods, without appeal to any special assumptions regarding the shapes of the response curves $q_a(t)$ and $q_v(t)$. Equation (7) for the mean transit time t holds for injections with general time courses, provided the normalized arterial and venous injection functions $i_a(t)$ and $i_v(t)$ are identical and that their first moments exist. When $i_a(t) \neq i_v(t)$, the more general equations of Reference 7 must be used in place of Equation (7).

Equation (7) for the mean transit time t may be given a simple geometric interpretation: the mean transit time is numerically equal to the area *between* the normalized arterial and venous response curves, $q_a(t)$ and $q_v(t)$, divided by the quantity $1 - q_\infty$. In this interpretation, the area between the two curves is taken as positive when $q_a(t) > q_v(t)$ and negative when $q_a(t) < q_v(t)$, so that t is equal to the algebraic sum of the areas, divided by $1 - q_\infty$. These areas are shown as the shaded regions in Figures 3 and 4, labelled with the appropriate algebraic sign.

The above results, which account for total or partial recirculation of tracer, also remain valid when there is *no* tracer recirculation. Thus, the results hold equally well for residue-detection models which, for simplicity, ignore recirculation effects altogether. In the present model, the steady-state content q_∞ of tracer within the detector field D will be zero in the absence of tra-

cer recirculation. (Even when there is no convective recirculation of tracer to D, the tracer input rates $\mathbf{x}(t)$ need not be zero since there may still be diffusive input fluxes.) When there is no recirculation of tracer in our model, then $q_v(t) = 0$ and $q_\infty = 0$, and a single arterial injection suffices. This is the model considered by Zierler.[1] Thus it may be seen that Equation (7) reduces, as expected, to the form given by Zierler[1] for measuring the mean transit time t by residue detection in the absence of tracer recirculation, viz.,

$$t = q_{0a}^{-1} \int_0^\infty q_a(t)\,dt = \int_0^\infty q_a(t)\,dt. \quad (8)$$

MEAN BLOOD FLOW

By the general volume theorem (or central-volume principle) of indicator dilution theory,[1,8,9] the first moment m_1 of the transit-time distribution, i.e., the mean transit time t of traced substance, is related to the geometric volume V of the system of interest and the flow rate F of traced substance through the system by the equation

$$m_1 = t = \lambda V/F. \quad (9)$$

Here, λ is the average[1,10] tissue-to-blood partition coefficient, defined as the steady-state ratio of the volume-average concentration of tracer in V to its concentration in traced fluid (e.g., blood plasma). Hence, on combining Equations (7) and (9), we obtain for the ratio of flow rate of traced substance to the volume of the system

$$\frac{F}{V} = \frac{\lambda(1 - q_\infty)}{\int_0^\infty [q_a(t) - q_v(t)]\,dt}. \quad (10)$$

The above result holds when there is recirculation of tracer to the system of interest h(t) and to adjacent perfused tissues within the field of view of a radiation detector used in residue detection, independently of the complexities of the recirculation paths and of the flow patterns within the detector field. Equation (10) is equally valid when the only system within the detector field is h(t), since in this case any recirculating tracer in adjacent perfused tissues will not influence the detector response.

DUAL-INJECTION ANIMAL EXPERIMENTS

At Washington University School of Medicine, numerous animal experiments designed on the basis of our dual-injection theory have been performed. Specific numerical results will appear in future publications as more data are accumulated and as we refine the computational procedures for implementing our equations. Here, we describe briefly the essential features of these experiments and give some indication of the agreement between blood-flow values predicted on our theory with those measured directly.

Figures 5–7 represent, schematically, some specific examples of the general configuration of Figure 2. Each diagram shows a particular vascular system of interest, h(t), (renal, myocardial, and cerebral) within the detector field of view, which also encompasses other vascular systems from which interfering radiations due to recirculating tracer may emanate. Recirculation, of course, also carries radiotracer into the systems of interest. In each of these situations, the system for which the transit-time distribu-

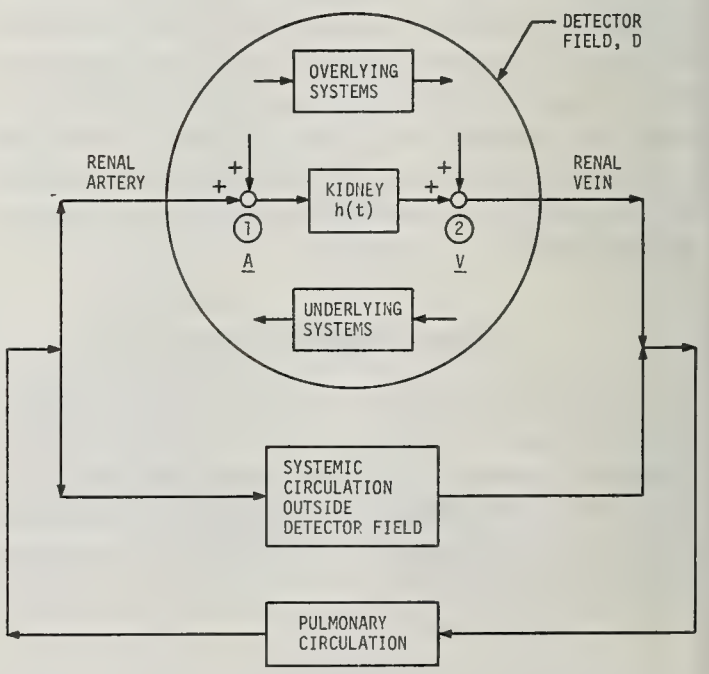


FIGURE 5.—Residue Detection with Recirculation for the Kidney.

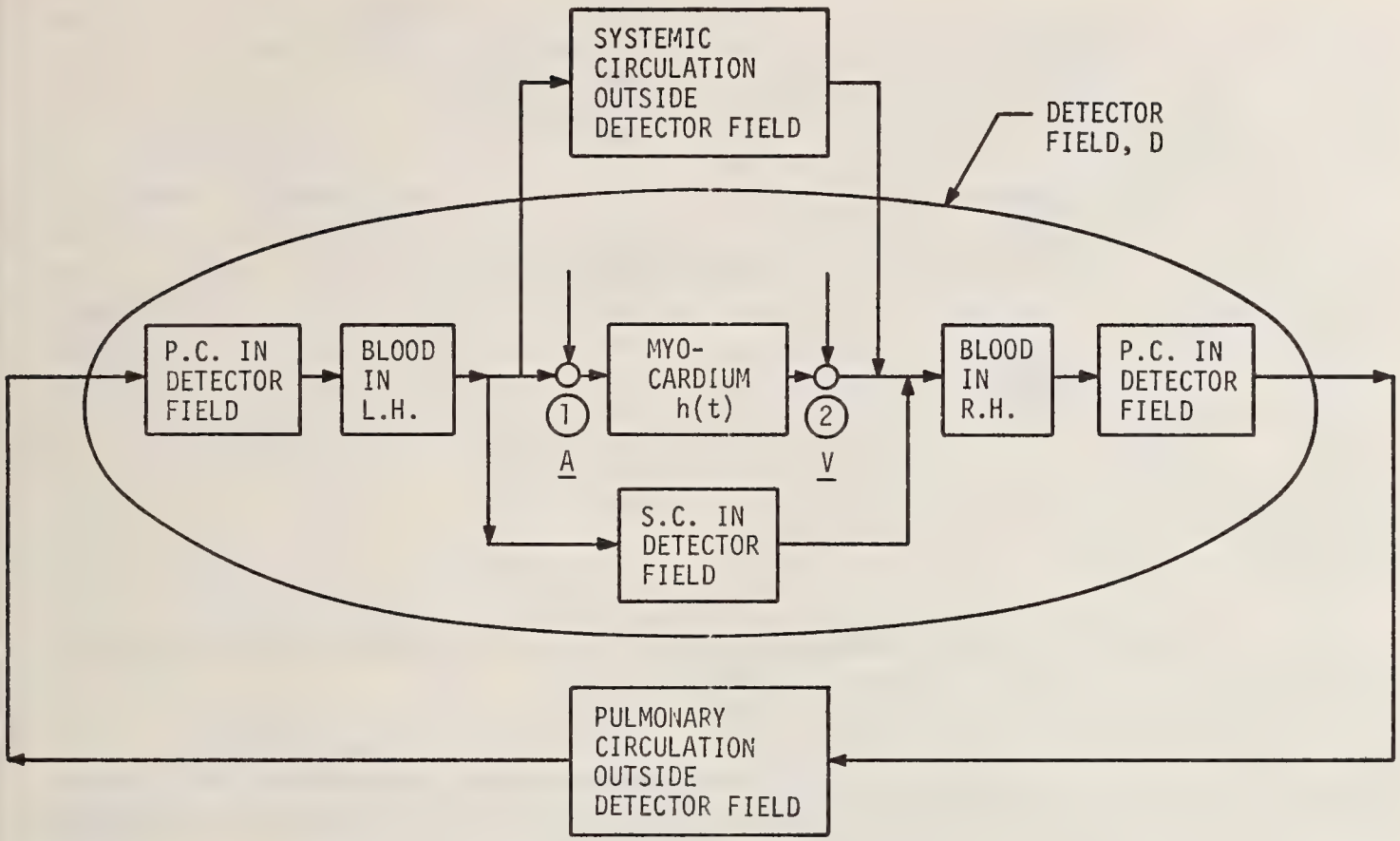


FIGURE 6.—Residue Detection with Recirculation for the Heart.

tion is obtained on our model is defined by the location of the sites for injection experiments *A* and *V*. In the Washington University dual-injection experiments, two radioisotopes have been employed: ^{15}O and ^{133}Xe. The first is used to label water which is then mixed with saline solution to form the injectate. This cyclotron-produced oxygen isotope is a positron-emitter whose short half-life of 124 seconds requires that it be prepared immediately prior to injection. Five-day ^{133}Xe, obtained commercially, is a gamma-emitter, and is dissolved in saline solution prior to injection. The annihilation radiation from ^{15}O and the gamma radiation from ^{133}Xe are monitored continuously as a measure of the amounts of tracer within the organ and adjacent tissues following injection. The detectors employed are collimated NaI(Tl) scintillation counters. Detector data are accumulated on magnetic tape using a LINC computer, which is also programmed to correct the data for physical decay of the isotopes and for radioactive background.

The responses (normalized to the injected doses) corresponding to the two injection stimuli are denoted $q_a(t)$ and $q_v(t)$, respectively. When there is little or no loss of tracer during the course of an experiment, both normalized responses approach a common steady-state value, denoted q_∞. These raw data are then processed as specified by Equation (10), yielding a value of blood flow relative to volume. For those experiments in which flow, volume, and the partition coefficients have been measured independently, agreement with the ratio predicted by Equation (10) has generally been to within 10% or less. These results are encouraging and allow us to place confidence in the applicability of our theory.

SUMMARY

We have developed a mathematical model applicable for external monitoring of radiotracer emissions when recirculation is *not* a late event and must be taken into consideration. Addition-

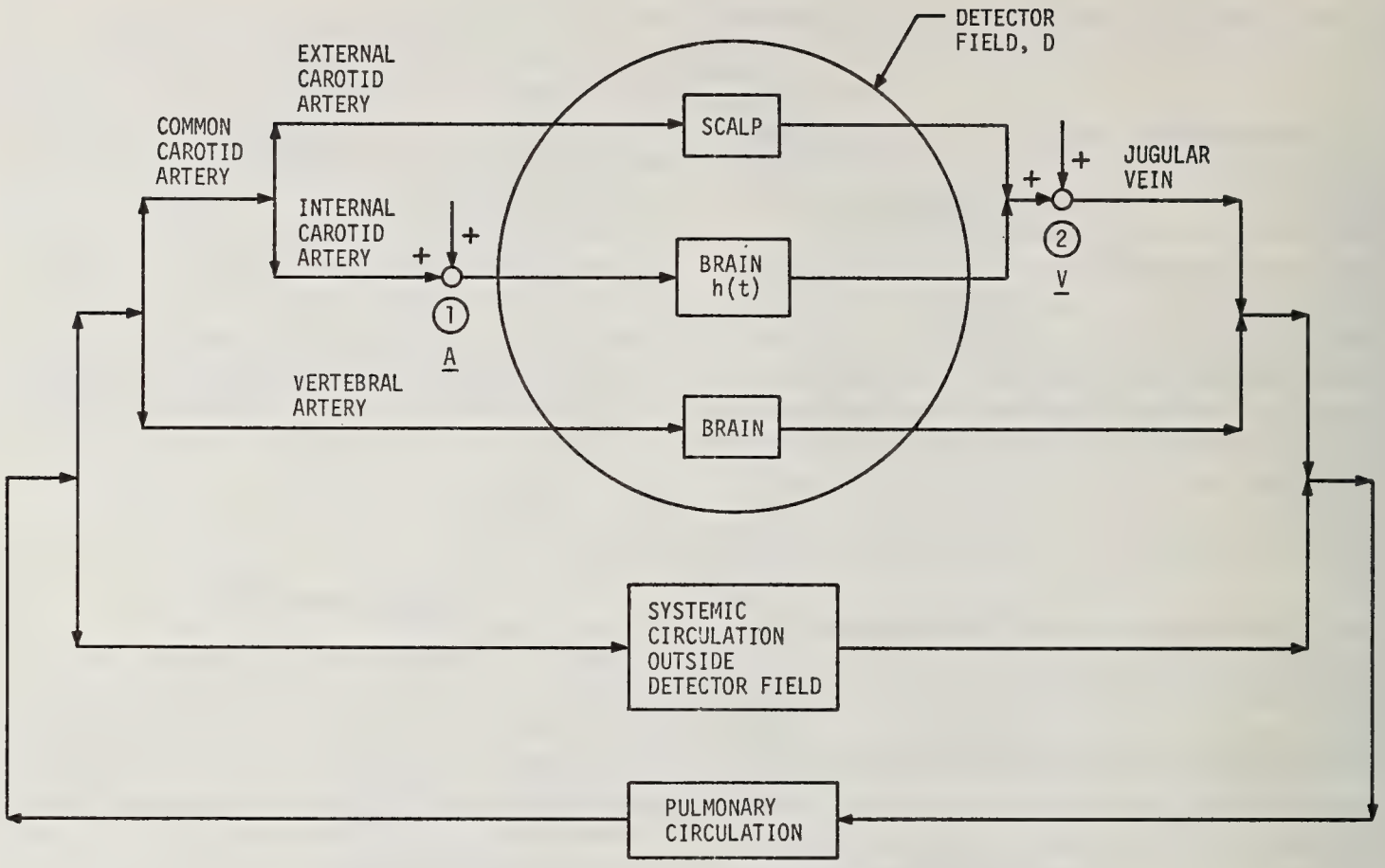


FIGURE 7.—Residue Detection with Recirculation for the Brain.

ally, the model accounts for interfering recirculation of tracer to adjacent perfused regions in the field of view of the detector. Central to the model is the use of two injections of tracer—one upstream (arterial) and one downstream (venous)—of the particular organ or region of interest. Thus, two residue curves are obtained. We have developed equations indicating how to employ the two residue curves in order to determine the mean transit time of tracer through the vascular system of interest, as well as higher moments of the transit-time distribution if they are desired. These equations are natural generalizations of Zierler's residue-detection method in that they do not depend for their validity on any model of mass transport within the system of interest. The numerical calculations implied by our equations need not rely on curve-fitting of the data. In addition to mean flow per unit volume, our method can yield compartmental parameters if these are appropriate and desired.

We describe briefly various animal experiments designed on the basis of our dual-injection theory, and give some indication of the agreement between blood-flow values predicted on our theory with those determined independently.

ACKNOWLEDGMENTS

We thank Dr. Jerome R. Cox, Jr. for his support and encouragement of our work. Drs. Maxine L. Rockoff and Peter E. Peters called our attention to the problem of recirculating tracer in blood-flow studies, and we thank them for their illuminating discussions. Drs. John O. Eichling, Judith M. Metzger, and Peter E. Peters have obliged us by testing the usefulness of our model and results in practice through blood-flow studies in patients and animals. These will be reported in later papers.

REFERENCES

1. ZIERLER, K. L. Equations for measuring blood flow by external monitoring of radioisotopes. Circ. Res. 16:309–321, 1965.

2. ZIERLER, K. L. The cardiovascular system. *In* Compartments, Pools, and Spaces in Medical Physiology, Bergner, P.-E.E. and Lushbaugh, C.C., editors, Clearinghouse for Federal Scientific and Technical Information, NBS, U.S. Dept. of Commerce, Springfield, Va., 1967.
3. ZIERLER, K. L. Circulation times and the theory of indicator-dilution methods for determining blood flow and volume. *In:* Handbook of Physiology, Vol. 1, Hamilton, W., and Dow, P., editors, American Physiological Society, Washington, D.C., 1962.
4. STEPHENSON, J. L. Theory of the measurement of blood flow by the dilution of an indicator. Bull. Math. Biophys. 10:117–121, 1948.
5. MEIER, P., and ZIERLER, K. L. On the theory of the indicator-dilution method for measurement of blood flow and volume. J. Appl. Physiol. 6:731–744, 1954.
6. PERL, W., EFFROS, R. M., and CHINARD, F. P. Indicator equivalence theorem for input rates and regional masses in multi-inlet steady-state systems with partially labelled input. J. Theor. Biol. 25:297–316, 1969.
7. LARSON, K. B., and SNYDER, D. L. Measurement of relative blood flow, transit-time distributions and transport-model parameters by residue detection when radiotracer recirculates. J. Theor. Biol. 37: 503–529, 1972.
8. STEPHENSON, J. L. Theory of measurement of blood flow by dye dilution technique. IRE. Trans. on Med. Electronics PGME-12, 82–88, 1958.
9. CHINARD, F. P., EFFROS, R., PERL, W., and SILVERMAN, M. Organ vascular and extravascular compartments in vivo. *In* Compartments, Pools, and Spaces in Medical Physiology, Bergner, P.-E.E. and Lushbaugh, C.C., editors, Clearinghouse for Federal Scientific and Technical Information, NBS, U.S. Dept. of Commerce, Springfield, Va., 1967.
10. PERL, W., and CHINARD, F. P. A convection-diffusion model of indicator transport through an organ. Circ. Res. 22:273–298, 1968.

DISCUSSION

QUESTIONER (UNIDENTIFIED): Does it matter what kind of radioactive isotope you use? Do you have one that you use regularly?

DR. LARSON: It has to be a radioisotope that can be detected externally, which means something generally not less than about 80 or 90 keV for the emitted electromagnetic radiation. We have used two radioisotopes in our experiments: ^{15}O and ^{133}Xe. The first is a cyclotron-produced positron emitter used to label water; the annihilation radiation from the attending positron-electron interactions has an energy of 511 keV. The second is dissolved directly in saline solution to form the injectate. The gamma radiation from ^{133}Xe has an energy of 83 keV.

MARVIN BLEIBERG, Woodard Research Corp., Virginia: In your introduction, you mentioned the intercept. Could that be accounted for by extending your line down to zero?

DR. LARSON: Well, this of course is what is done very often. An arbitrary extrapolation, an exponential extrapolation, is usually made, and this method is not subject to too much error if recirculation is a fairly late event. If it is an early event, the kind of extrapolation that you are suggesting is very uncertain.

DR. BLEIBERG: What are some of the equations you use when you're calculating the intercept?

DR. LARSON: May I write them on the blackboard, Mr. Chairman?

CHAIRMAN WARNER: Yes.

DR. LARSON: First, let me begin by writing the conventional equation for mean transit time. This integral [Equation (8)] is the area under an arterial washout curve, $q_a(t)$, say. The formula for mean transit time simply says that you find the total area under the arterial curve and divide by the amount you injected [Equation (8)]. This expression holds only if tracer does not recirculate, and is an approximation otherwise. When there *is* recirculation of tracer (and normally there always is some, to a greater or lesser degree), the arterial residue curve may not fall to zero. In fact, if there is no loss of tracer at all, it will come down to a nonzero steady state as I indicated in one of the slides (Figure 3). I assume your question, Dr. Bleiberg, refers to the method of calculating the area under the residue curve when there are tracer-recirculation effects. The conventional approximation used in practice is to plot the falling portion of the curve on semilogarithmic paper, trying to discern a straight line over some portion of it. The early portion of the curve is presumably free of later recirculation effects, and so, hopefully, when extrapolated out along the time axis, describes the curve that would have been observed after very long times if there were no tracer recirculation. To get the area, one performs the integration analytically, using the time constant obtained from the slope of the straight-line segment of the semilogarithmic plot. Unfortunately, small errors in the

estimate of the time constant can lead to large errors in the calculated area. I think that you can readily appreciate the uncertainties inherent in this approach.

Now, our modified recirculation model says that in order to compute the mean transit time, you have to make an additional injection which gives another response curve you can call $q_v(t)$. This curve represents the actual quantity of tracer in the detector field of view due to the venous injection. The modified formula reads like this: [Equation (10)]. Here, q_∞ is the steady-state value that the venous and arterial curves approach. If each is normalized to the amount injected, they will both approach the common value q_∞. As you can see from this formula [Equation (10)], the mean transit time on our model is related not to the area *under* the arterial curve, but to the area *between* it and the venous curve. Then, of course, by the central-volume principle, one can compute relative blood flow [Equation (9)].

CHAIRMAN WARNER: I don't really understand the basis for your saying that you're not dependent upon getting good mixing though. You're measuring instantaneous differences in concentration in two locations and . . .

DR. LARSON: I think what I said was that our model makes no assumptions as to tracer-transport mechanisms within extravascular tissue. Our equations are derived on the basis of tracer conservation alone. They say nothing at all about how tracers diffuse within the tissues.

CHAIRMAN: No, but you're going to have to. In order to deduce what is happening to the blood, you've got to know how the tracers are distributed in the blood, don't you?

DR. LARSON: I'm sorry, Mr. Chairman; I did not realize you were referring in your previous question to mixing in the blood. One of the assumptions of the central-volume principle [Equation (9)] is that there is complete mixing of tracer with the blood at the arterial inflow of the vasculature of interest. However, in order to compute the mean transit time on our model, we need no assumptions about the degree of tracer mixing anywhere else, whether inside or outside the detector field of view.

QUESTIONER (UNIDENTIFIED): I take it that these two injections are made at two different times.

DR. LARSON: Yes, they can be. In our experiments, we have made them sequentially. I suppose there is no reason they couldn't be made simultaneously if you had two channels for resolving the response curves.

QUESTIONER: Two different isotopes?

DR. LARSON: Yes—two different radioisotopes labeling the same tracer substance.

D. B. JACKSON, Abbot Laboratories, North Chicago: Does $\lambda V/F$ divided by the mean transit time equal one?

DR. LARSON: Yes, because by the central-volume principle, the mean transit time is equal to $\lambda V/F$.

DR. JACKSON: No, what's the λV mean?

DR. LARSON: λ is the average partition coefficient. We define this to be the ratio of the concentration of the tracer in the tissue to the concentration in the blood.

DR. JACKSON: What is V?

DR. LARSON: V is the geometrical volume in which the tracer is distributed.

PULSED DOPPLER ULTRASONIC FLOWMETRY

D. Y. Loisance, J. P. Lenriot and P. A. Peronneau*

A pulsed ultrasonic velocimeter which permits profile analysis of the instantaneous and mean blood velocity, has been used for the study of the portal and hepatic arterial blood flow in normal dogs and on a model of the human portal hypertension. Changes following the completion of a Side to Side Portacaval Shunt (SS–PCS) have been monitored. The directly measured volume flow value Q1 and the value Q2 computed from the mean velocity profile have been compared. In normal dogs, after completion of the SS–PCS, the hepatic arterial flow Q2 was doubled but the flow pattern was unchanged. The portal vein flow rate Q2 was reduced and the velocity profile showed a bidirectional flow pattern. On the experimental model of portal hypertension, the hepatic arterial flow, already increased by the ligation of the supra-hepatic veins, was still increased to higher values after SS–PCS. The portal vein, downstream from the shunt, was acting as an outflow tract for the liver. Comparative studies of the flow rate values Q1 and Q2 showed that the monitoring of the Q1 value only can be misleading in complex flow patterns such as in the portal vein downstream from the SS–PCS. This experiment points out the necessity of a study of the blood flow pattern for an accurate assessment of the volume flow rate.

INTRODUCTION**

The qualitative study of the blood flow pattern in a vessel for an accurate measurement of the blood flow rate must be emphasized. Both techniques of instantaneous blood flow measurement, the electromagnetic and the ultrasonic flowmetry, require such an analysis.[1] As a matter of fact, the relation between the electromagnetic signal and the average blood velocity is linear only in the case of axially symmetrical flow pattern. The significance of the ultrasonic signal, on the other hand, is difficult to assess because of the great number of different reflected echoes and the unknown direction of the velocity vector of every red cell.[1]

* Centre d'Etude des Techniques Chirurgicales (Head : Professeur J. P. Cachera), Hôpital Broussais, Paris.

** Work done with support from la Délégation Générale à la Recherche Scientifique et Technique (Paris).

The pulsed Doppler ultrasonic velocimeter designed by P. Peronneau[2] permits blood flow studies of a qualitative (blood flow velocity profile) and quantitative (blood flow velocity and blood flow rate) nature. The description of the velocity profile in the portal vein and the hepatic artery of dogs in various experimental conditions is discussed here.

EXPERIMENTATION

Briefly, the basic principle of the device is as follows. A single crystal operates alternatively as transmitter and receiver. The ultrasonic emission is pulsed (frequency 8 MHz). In the interval between two pulses the crystal receives the echoes reflected from the vascular walls and the red cells. Consequently, the internal diameter of the vessel is measured and an electronic gate can select the reflection due to only the red cells at a given distance from the transducer and in a given volume of flow.

The practical technique of the blood flow study is as follows. After setting the ultrasonic probe on the vessel, the internal diameter is measured. The instantaneous (V) and mean (V) velocities are measured according to two different techniques: 1. with the observation gate extended to encompass the entire internal diameter the average velocity Vd of the flow taken as a whole results. 2. by diminishing the width of the electronic gate to as little as .765 millimeters, it is possible to scan from the near to the far wall of the vessel. This narrow gate selects the velocity signal Vi from a restricted area of the flow. The resultant figures establish the velocity profile. The volume flow rate is calculated in two different ways: 1. the flow value Q_1 as the product of the average velocity Vd and the section of the vessel defined by the measured internal diameter; 2. the flow value

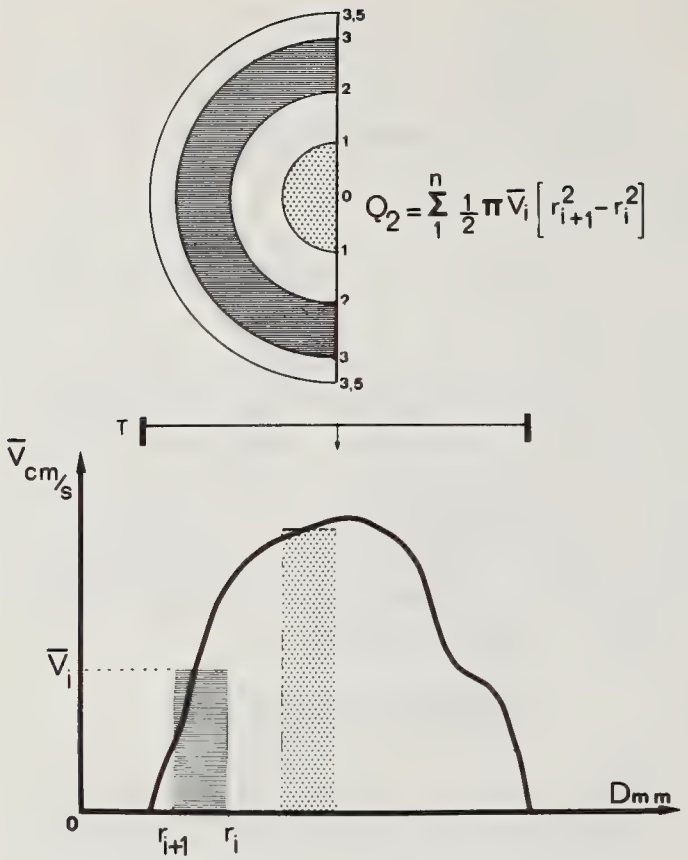


FIGURE 1.—Schematic representation of the hemi-annular averaging computation of the blood flow rate from the mean velocity profile. The velocity Vi at a distance r_i from the vessel axis is assumed to be the average velocity on half a circle of radius r_i, as represented on a section of the vessel (above).

Q_2 is the result of the hemiannular averaging computation of the mean velocity profile (Fig. 1). The degree of asymmetry of the mean velocity profile is expressed in percent as the difference between the computed flow for each half of the mean velocity profile divided by the total flow Q_2.

The experiment was carried out on normal dogs (mean body weight 23.5 ± SD 1.1 Kg), anesthetized, intubated and mechanically ventilated. A laparotomy was performed. The ultrasonic probes were placed on the hepatic artery and the portal vein. Snares were placed around the supra-hepatic veins. The hepatic arterial, portal, wedged supra-hepatic pressures were continuously monitored. Two groups of dogs were compared: normal dogs (n=15) and dogs with increased intra-hepatic resistances, obtained by the ligation of the right and the left supra-hepatic veins (n=15). The changes in pressures and flow due to the completion of a wide and distally located side to side portacaval shunt were studied.

RESULTS

1. *Flow pattern.* The hepatic arterial flow profile is never symmetrical in the different experimental conditions. The degree of the asymmetry is related to changes in the direction of the hepatic artery. The highest velocities were located in the outer side of the vessel's curvature. The increase of the arterial velocity Vd following the ligation of the supra-hepatic veins did not alter the velocity distribution in the hepatic artery. After side to side portacaval shunt, in both normal dogs and dogs with ligated supra-hepatic veins, the average velocity Vd was increased but the shape of the velocity profile unchanged.

In the portal vein, very different velocity profiles were observed in the various experimental conditions. On the normal dog, in the plane defined by the portal vein and the gastro-duodenal vein two different aspects were observed with an equal frequency: a curved profile (Fig. 2A) and a double humped profile (Fig. 3 left). This latter shape was caused by the arrival of the gastroduodenal vein upstream from the ultrasonic probe. It reveals a stream line effect in the portal vein flow. The ligation of the supra-hepatic veins reduced the average velocity Vd but did not alter the shape of the velocity profile of the portal flow. The completion of the side to side portacaval anastomosis decreased the average velocity Vd on both groups of dogs: in normal dogs, as Vd remains directed to the liver, the velocity profile showed a bidirectional flow pattern (Fig. 2D). Two different streams of opposite direction, hepatopetal and hepatofugal, were juxtaposed in the vein. On dogs with ligated supra-hepatic veins, both average velocity Vd and segmental velocities Vi were reversed, directed from the liver towards the shunt. The shape of the reversed mean velocity profile was then flattened (Fig. 2C).

2. *Blood flow rate.* In the normal dog, the mean portal flow value Q_2 was 303 ± SD 35 ml/min and the hepatic arterial flow rate Q_2 was 107, ± 8 ml/min. In the group of dogs with

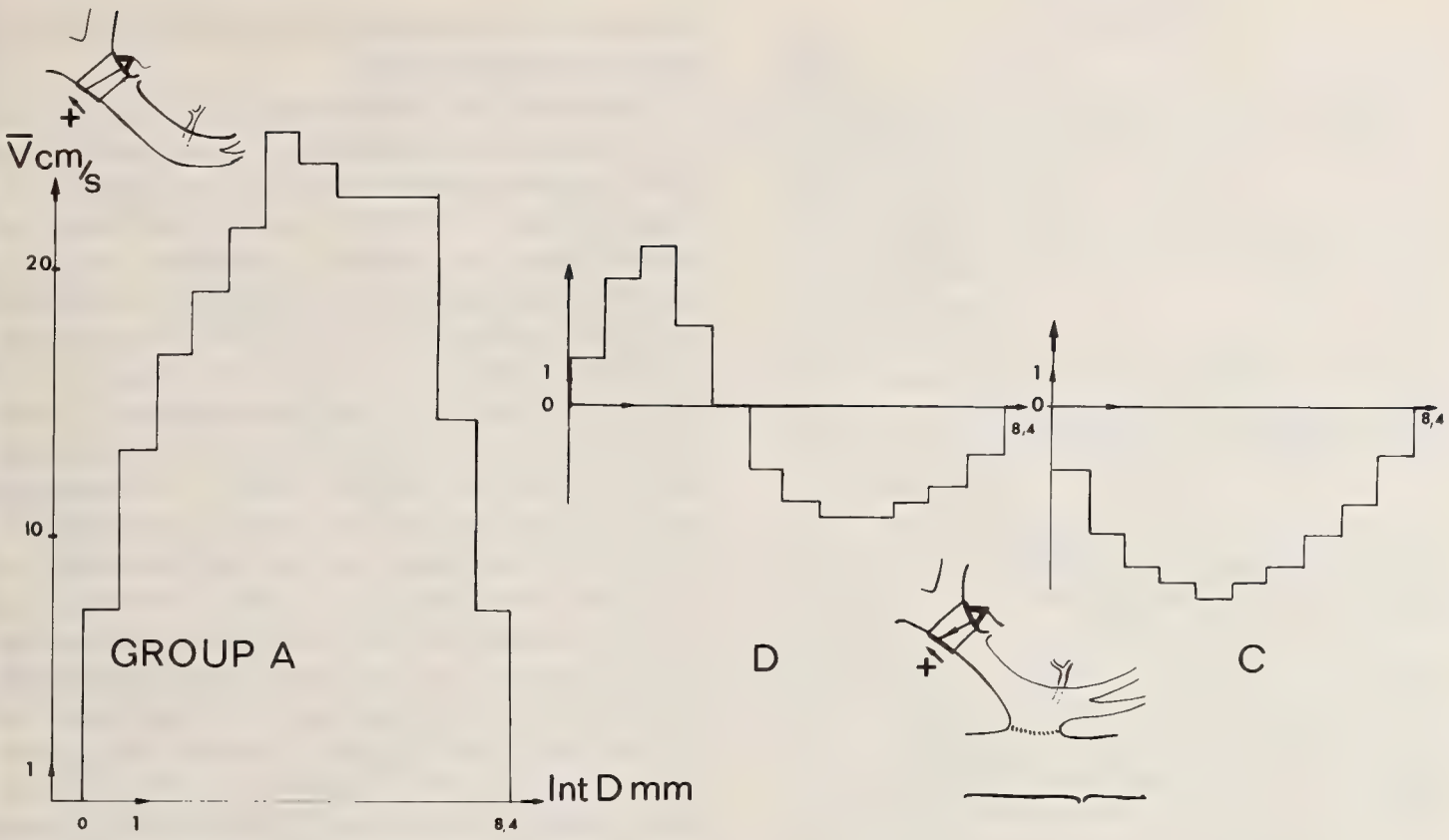


FIGURE 2.—Portal velocity profile in normal dog (A), after SS PCS in normal dog (D) and in dogs with ligated supra-hepatic veins (C).

ligated supra-hepatic veins, as the intrasinusoidal pressure and the free portal pressures were increased (respectively by 135 ± 13% and 75 ± 32%), the mean portal flow rate Q_2 decreased by 36 ± 5% and the arterial flow rate rose by 19 ± 10%.

The completion of the side to side portacaval shunt on the first group increased the hepatic arterial flow rate by 99 ± 21%, and decreased the portal flow rate by 79 ± 5% as measured downstream from the shunt. The portal flow was then still directed towards the liver. In the second group, the arterial flow rate rose to 196 ± 21 ml/min (i.e., increased by 152 ± 8% as compared to its value prior to the shunt). The portal flow rate was decreased by 122 ± 9% ($Q_2 = -46 \pm 27$ ml/min). The portal vein then acted as an outflow tract for the liver, and 23% of the hepatic arterial flow was diverted from the liver to the side to side portacaval anastomosis.

3. *Comparison of the* Q_1 *and* Q_2 *flow rate values.* In every experimental condition the directly measured value of the flow rate Q_1 was higher than the computed value Q_2. The difference in the case of a normal blood flow pattern (25% for the hepatic arterial flow, 30% for the portal vein flow) was considerably increased in complex flow patterns, such as in the portal vein, after the completion of the side to side portacaval shunt. In such conditions, the flow rate value Q_7 was higher than the computed value Q_2 by as much as 80%.

COMMENTS

The experimental data presented here and our previous studies[3,4,5] emphasize the practical and theoretical problems of the instantaneous blood flow measurement and the definite advantages of the pulsed Doppler ultrasonic flowmetry.

The simplicity of the present technique must be emphasized. Bulk and mass of the ultrasonic transducer have been reduced when compared to the electromagnetic probes. The mechanical hazards such as the displacement of the transducer and the kinking of the vessel are avoided.

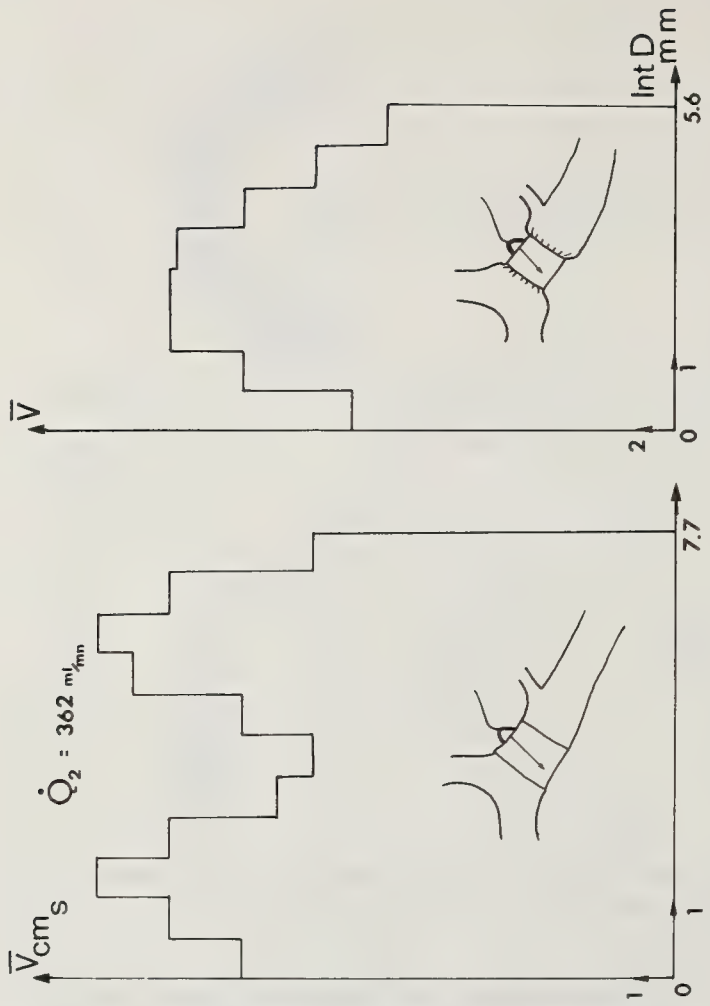


FIGURE 3.—Portal flow pattern changes during the chronic implantation of an ultrasonic probe: velocity profile as measured immediately after surgery (left) and at one week postoperatively (right).

Furthermore, the close and constant contact of the ultrasonic probe on the vessel is required only a short part of the vessel. So, the correct position of the transducer is easily obtained, without the complete dissection of the vessel's circumference.

The assessment of the internal diameter of the vessel on the echogram avoids the complete dissection of the thick nervous plexus surrounding the hepatic artery. On the other hand, the use of this actual internal diameter for the computation of the blood flow rate is more accurate than the use of a diameter value suggested by the size of the flow probe, as is done in the electromagnetic flowmetry. The consequent overestimation of the measured average blood flow rate in the latter case can be considerable, only for this reason,[6] especially in the case of an abnormal and unknown thickening of the vessel walls.

The comparison between the value Q_1 and Q_2 of the flow rate emphasizes the necessity of a flow pattern analysis prior to the computation of the volume flow rate. It must be recalled, that the accuracy of various techniques used to measure instantaneous volume flow rate depends on some assumptions about the shape of the velocity profile. For example, the linearity of the electromagnetic flow measurement is good in the case of an axisymmetrical flow pattern. In complex flow patterns such as downstream from a side to side portacaval anastomosis, and in very asymmertical flow patterns such as in a curved vessel, such cause of error should not be forgotten. The limitations implied by the ultrasonic illumination of a single diameter only must not be forgotten, nor the errors due to the fact that a single ultrasonic transducer does not define the direction of the velocity vectors. Recent development of the method[7] have made possible such studies. This problem is particularly interesting in complex flow patterns such as in the side to side portacaval anastomosis itself, as suggested by the shape of the mean velocity profiles monitored in a sagittal and axial plane (Fig. 4).[8]

Our present data point out the need for a non-invasive method of blood flow measurement. The studies performed within the first hours following the completion of the side to side portacaval anastomosis on an anesthétized and open abdomen dog show only the initial evolution of the hepatic circulation changes. The implantation of paravascular flow probes induces inflammatory changes responsible for the marked changes of the flow pattern (Fig. 3). A catheter tip velocity probe is presently under investigation.

SUMMARY

A pulsed Doppler ultrasonic velocimeter has permitted the studies of blood flows of a qualitative and a quantitative nature, in normal dogs and on an experimental model of the human portal hypertension. Blood flow patterns and volume flow rates in the portal vein and in the hepatic artery have been described before and

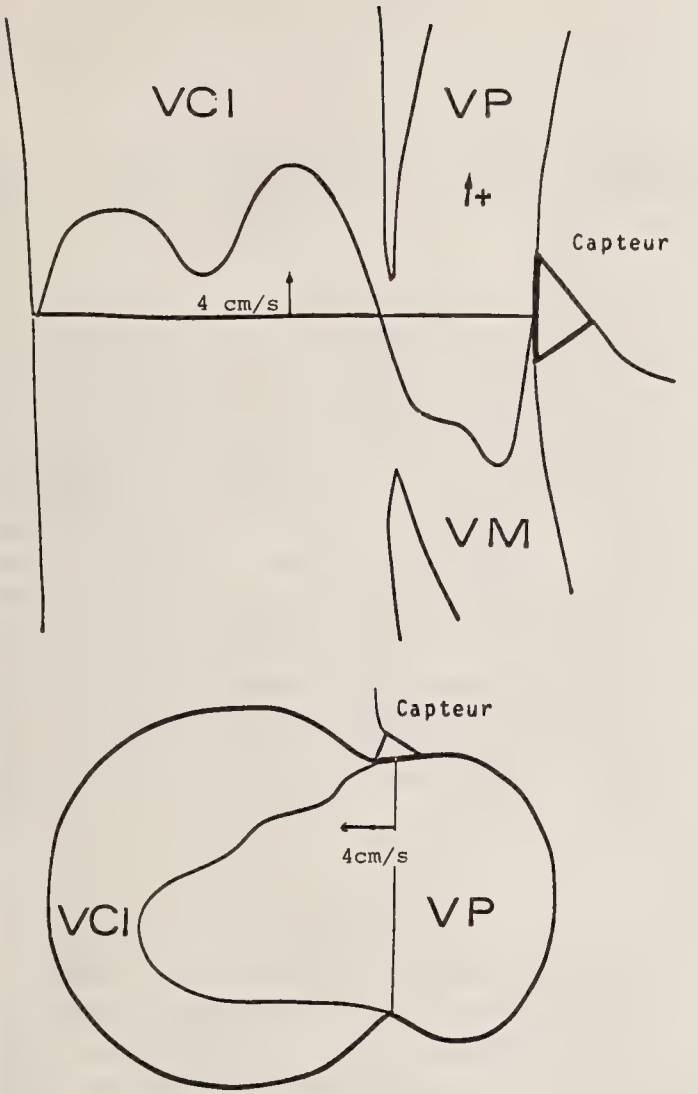


FIGURE 4.—Blood flow velocity profile through the SS PCS itself, in a sagittal (above) and axial (below) plane. Capteur = probe.

after the completion of a side to side portacaval anastomosis. The main advantages of the technique have been briefly described.

REFERENCES

1. PERONNEAU, P., HINGLAIS, J. La mesure continue du débit sanguin instantané. La Presse Médicale, Paris, 79:1225–1226, 1971.
2. PERONNEAU, P., LÉGER, F., HINGLAIS, J., PELLET, M., SCHWARTZ, P. Vélocimètre sanguin à effet Doppler à émission pulsée. L'onde Electrique, Paris, 50: 369–389, 1970.
3. LOISANCE, D., PERONNEAU, P., PELLET, M., LENRIOT, J. P. Etude de la circulation hépatique artérielle et portale au moyen d'un vélocimètre à effet Doppler ultrasonore. La Presse Médicale, Paris, 79: 1227–1230, 1971.
4. LOISANCE, D., PERONNEAU, P., PELLET, H., HINGLAIS, J. Etude peropératoire des pontages aorta-coronariens par débitmétrie ultrasonore. La Presse Médicale, Paris, 79:2130–2133, 1971.
5. LOISANCE, D., LENRIOT, J. P., KAGAMI, H., PERONNEAU, P. Effect of respiration on portal vein flow patterns with or without side to side portacaval shunt. Presented at the 7th Congress of the European Society for Experimental Surgery, Amsterdam, Holland, abst. p. 183, April 11–14, 1972.
6. SADONY, V., HAGL, S., NEUHAUS, K., HINGLAIS, J. Dynamics of coronary artery circulation studied by ultrasonic flowmetry. Presented at the 6th Congress of the European Society for Experimental Surgery, Helsingborg, Sweden, abst. p. 157, April 27–29, 1971.
7. PERONNEAU, P., PELLET, M., XHAARD, M., HINGLAIS, J. Pulsed Doppler ultrasonic blood flowmeter. Real time instantaneous velocity profile. Symposium on the flow, Pittsburgh, Pa., abst. p. 146, May 10–14, 1971.
8. LOISANCE, D., PERONNEAU, P., PELLET, M., LENRIOT, J. P. Hepatic circulation after side to side portacaval shunt in dogs. Velocity pattern and flow rate changes studied by an ultrasonic velocimeter. Surgery, January 1973.

IMPLANTABLE ULTRASONIC BLOOD FLOWMETERS*

James D. Meindl*

Accurate measurement of pulsatile blood flow can be achieved on a chronic basis in research animals through the use of totally implantable ultrasonic flowmeters. The continuous wave Doppler flowmeter provides a technique for measurement of flow velocity at a particular location such as the center of the lumen; the pulsed Doppler flowmeter is useful for measurement of flow velocity distribution or profile across the vessel and lumen diameter and hence volume flow. Both instruments can be electronically precalibrated and exhibit no baseline or scale factor changes during chronic experiments. Custom designed silicon monolithic integrated circuits offer significant advantages in reduced size and power drain as well as improved reliability in these instruments.

INTRODUCTION*

Accurate measurement of pulsatile blood flow is of major importance in cardiopulmonary research and development. It is particularly useful to perform this measurement on a chronic basis in research animals under a variety of normal and abnormal conditions. The most promising instruments available for this purpose are ultrasonic flowmeters. This paper briefly reviews their principles of operation as well as their primary capabilities and limitations. In addition, a discussion is presented of two new chronically implantable ultrasonic flowmeters which are designed using unique families of silicon monolithic integrated circuits. Following initial applications in animals, it is conceivable that these implantable flowmeters may be useful in heart surgery patients. For example, by attaching a remotely removable transducer to the aortic wall and connecting it to a subcutaneous electronics package, accurate monitoring of cardiac output (i.e., instantaneous volume of blood flow) might be safely accomplished during the most critical phase of recovery.

* Department of Electrical Engineering, Stanford University, Stanford, California 94305.

** This investigation was supported by the U. S. Public Health Service Research Grant 1-PO1-GM17940-01.

CHRONIC PULSATILE BLOOD FLOW MEASUREMENT

The most desirable features of a chronically implantable blood flowmeter are listed in Figure 1. To begin with, the instrument should exhibit no measurement ambiguity; it should measure a well defined physiological quantity to a specific degree of accuracy. Ideally, it should require no calibration *in vitro* or *in vivo* by the user; it should come to him electronically precalibrated by the producer. During chronic experiments, the instrument should exhibit no significant changes in its baseline or its scale factor. For implantable instruments, small size is an obvious necessity and low power drain is essential to long operating life. To avoid trauma, non-constricting non-erosive flow transducer cuffs are necessary. Finally, to assure full ex-

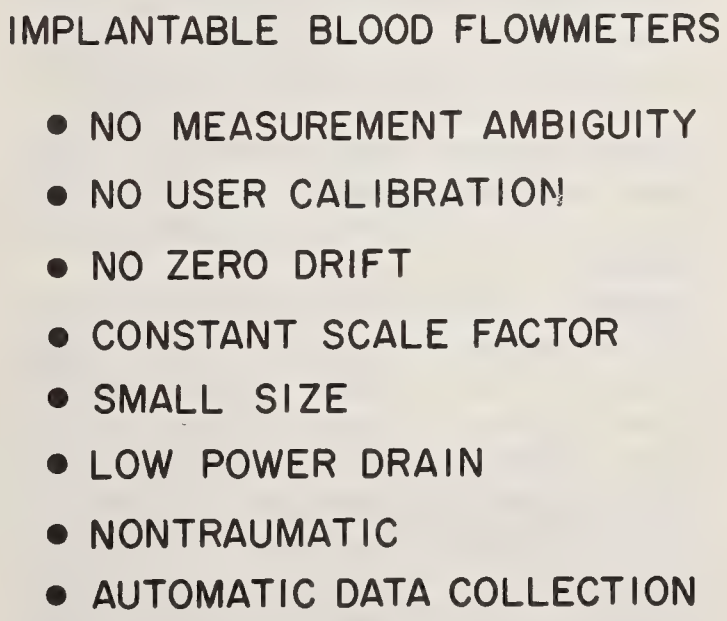


FIGURE 1.—Major desirable features of chronically implantable blood flowmeters.

ploitation of an implantable instrument, it should be immediately adaptable to automated data collection.

Electromagnetic Flowmeters

For many years the most widely used instrument for measurement of pulsatile blood flow has been the electromagnetic flowmeter.[1,2] Recently a battery operated version of this instrument was announced.[3] The most attractive feature of the electromagnetic flowmeter is that the "flow induced" voltage (e) between its electrodes is proportional to the average velocity of blood (v) flowing in a circular vessel of lumen diameter (D). Assuming axial flow symmetry and a uniform magnetic field intensity (B), solving Maxwell's equations for electromagnetic field yields

$$e = [SBD]\ \bar{v} \qquad (1)$$

where the sensitivity (S) depends on vessel wall thickness, the tensor conductivity of the wall and the blood conductivity.[4] If the factor (SBD) is known, (1) clearly indicates that the electromagnetic flowmeter provides a measurement of average blood velocity (v) only; to convert this information unambiguously to instantaneous volume of flow (Q) an entirely independent measurement of lumen diameter (D) is essential since

$$Q = \pi\ (D/2)^2\ \bar{v} \qquad (2)$$

Considering the factors which influence S, the product (SBD) can be determined accurately only by the user through *in vivo* calibration of a given flow transducer via a process such as excisting the vessel and collecting a known amount of blood in a given interval of time. Electronic calibration of the instrument is not feasible.

Baseline or zero drift is one of the most difficult problems encountered in electromagnetic flowmeters.[2,5] The principal cause of zero drift in most instances is unwanted voltages induced within the blood and the vessel wall by the sine wave or square wave excitation of the field coils of the flowmeter. To appreciate this problem, one might think of a field coil as an inductor imbedded in a lossy conductive medium consisting of the blood and the vessel wall. Eddy currents induced in this medium by the alternating excitation of the field coil persist for indefinite periods; the potentials they generate between the electrodes of the cuff constitute an unpredictable source of baseline shift which is particularly obscure in chronically implanted transducers. Leakage currents between the field coils and the input electrodes as well as other factors[5] also give rise to baseline shift. Consequently, to establish the approximate baseline it is necessary to clamp the vessel at both ends of the flow cuff in order to insure that there is no movement of blood within the cuff. In addition to baseline shifts, scale factor changes may occur during chronic implantation of a flow cuff due to variations in sensitivity (S) resulting from changes in vessel dimensions and tensor conductivity as well as blood conductivity as previously discussed.

Ultrasonic Transit Time Flowmeters

The difference in effective velocity of sound pulses propagated alternately upstream and downstream through moving blood is a measure of blood velocity that has been used as the basis of an ultrasonic blood flowmeter.[6] The difference in transit times upstream and downstream is

$$\Delta t = \frac{2\ L\ \bar{v}_p\ \cos\ \theta}{c^2} \qquad (3)$$

where L is the distance between transducers, $\bar{v}_p$ is the average flow velocity along the path of sound, c is velocity of sound in blood and α the angle between the blood velocity and sonic vectors.[7] An approximation implicit in (3) if it is to be used to describe an actual blood vessel is that the length of the sound path in the vessel wall and surrounding tissue is very small compared to the path length in blood. However, the key factor to recognize is that $\bar{v}_p$ in (3) is not the velocity averaged over the entire lumen area (i.e., $\bar{v}$) but rather the velocity averaged along the linear path of sound between the two transducers. Since various velocity profiles or average velocities $\bar{v}$ may give rise to the same $\bar{v}_p$ there is clearly an ambiguity present in attempting to measure volume flow.[8] Consequently, empirical calibration by the user is necessary if one attempts to use this device to estimate volume of flow.

To achieve a practical transit time flowmeter, differences in acoustic transit times on the order of 10^{-10} sec must be measured.[9] This time interval is extremely small and the instrumentation required to measure it tends to be complex, bulky, drift-prone and expensive. Consequently, techniques have been developed for measuring the phase difference $\Delta\phi$ between the upstream and downstream sound pulses.[10,11] In this case, it appears that the baseline or zero flow output of the instrument depends on accurately setting the phase relationships of the two transmitter and receiver channels.[11] This is done initially by adjusting a tank circuit but cannot be checked during implantation when, for example, changes in transducer impedances and hence channel phase shifts cause zero drift.

Continuous Wave (CW) Doppler Ultrasonic Flowmeters

A second type of ultrasonic blood flowmeter is based upon the Doppler effect. In this instrument a beam of ultrasonic energy is transmitted through the wall of a blood vessel where it is scattered by the red cells of the bloodstream. The scattered energy, which is shifted in frequency according to the Doppler principle, is detected by a second transducer on the opposite side of the vessel.[12,13] The Doppler frequency shift (f_d) in the scattered ultrasound is given by

$$f_d = f_i - f_s = 2\frac{v}{c} f_i \cos\alpha \qquad (4)$$

where f_i is the incident frequency, f_s the frequency of the scattered sound, v the blood velocity, c the velocity of sound in blood, and α the angle between the blood velocity and incident sound vectors. Because blood velocity is a function of radial position within the lumen, (4) represents only a description of the physical principle of operation of the CW Doppler flowmeter and not necessarily its quantitative behavior. The design details of the transducers and the demodulator determine whether this instrument provides an estimate of flow velocity at a particular position or average velocity over the lumen (i.e., volume flow) or neither.[14,15]

To measure blood velocity at the center of the lumen the CW Doppler flowmeter transducers should be designed such that the diameter (ΔD) of the volume of intersection of the two transducers beam patterns is small compared with the lumen diameter (D) as illustrated in Figure 2. In this case v is effectively a constant and (4) accurately describes f_d as essentially a single line spectrum. Consequently, a simple zero crossing demodulator (which in effect computes the second moment of the scattered signal power density spectrum) provides an accurate estimate of velocity at the center of the lumen.[14,15] The benefits to the user of measuring central lumen blood velocity are significant. It is an unambiguous measurement; the measuring instrument can be electronically precalibrated with a high degree of absolute accuracy; and, the measurement is virtually immune to zero drift and changes in scale factor. In addition, estimates of volume flow for a particular vessel can be made by assuming a flow profile and a lumen diameter or by empirical calibration of the instrument.

In contrast, to measure volume flow with a CW Doppler flowmeter specifically designed for the purpose, three distinctly different requirements must be met:[14,15]

(1) the entire lumen cross-section must be uniformly illuminated by the transmitting and receiving transducers;

(2) the demodulator must compute the first moment of the power spectral density function of the Doppler shifts S(f) such that

$$\bar{f}_d = \frac{\int_{-\infty}^{\infty} fS(f)\,df}{\int_{-\infty}^{\infty} S(f)\,df} = 2\frac{\bar{v}}{c} f_i \cos\theta \qquad (5)$$

where $\bar{f}_d$ is the average frequency in S(f) and $\bar{v}$ is the average blood velocity; and

(3) lumen diameter must be measured by some independent technique to compute volume flow in accordance with (3).

Pulsed Doppler Ultrasonic Flowmeter

In principle, the pulsed Doppler ultrasonic flowmeter is an elegant extension of the CW Doppler velocity flowmeter. In this instrument a short burst of ultrasonic waves is transmitted through the wall of a blood vessel where it is scattered by the red cells of the bloodstream.

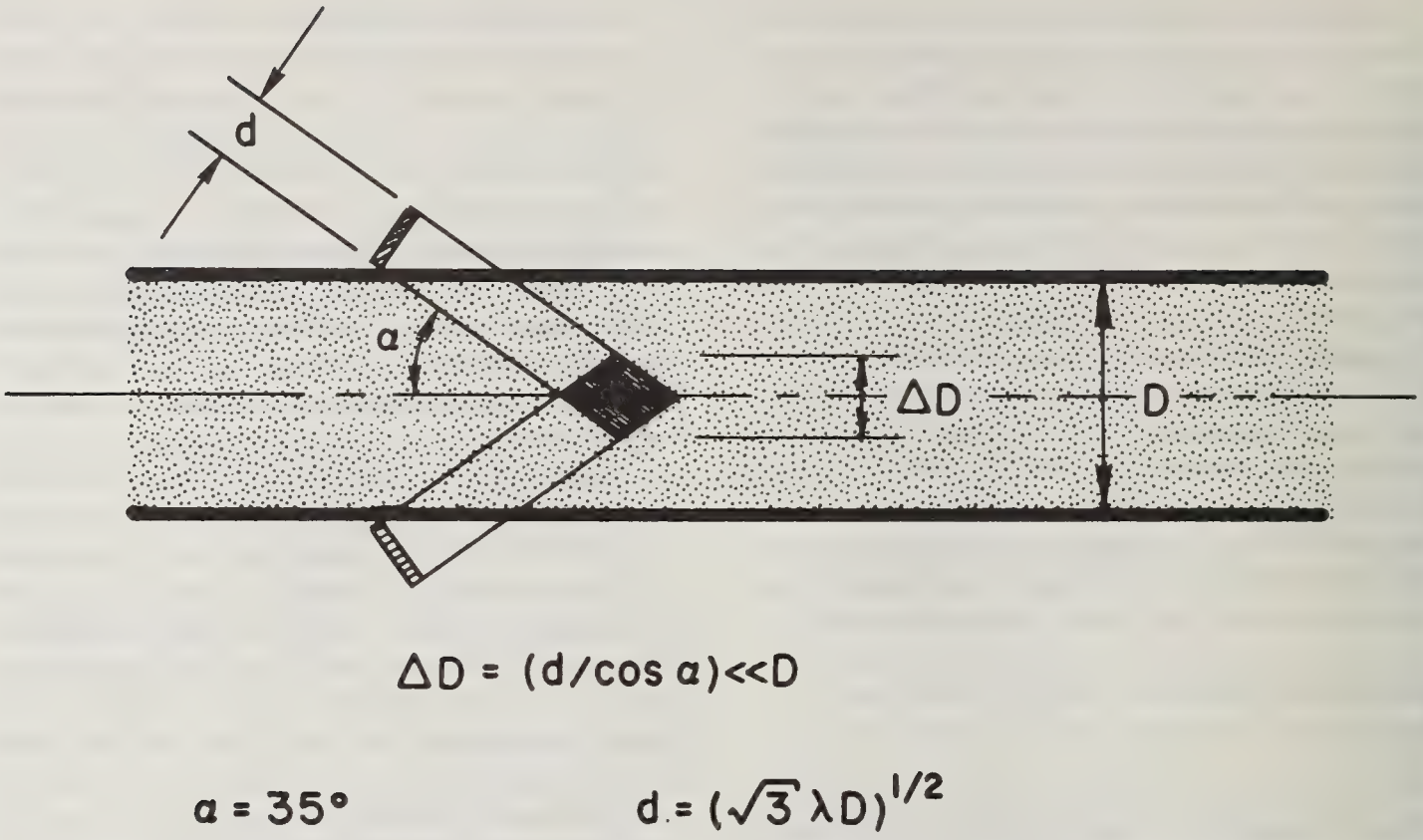


FIGURE 2.—Schematic diagram of CW transducers and beam patterns including condition for measurement of blood velocity at the center of the lumen ($\Delta D << D$) and optimum transducer design ($\alpha = 35°$ and $d = \sqrt{3}\lambda D)^{1/2}$ for meeting this condition.[14]

The scattered energy, which is Doppler shifted in frequency, is detected by the same transducer to provide a measure of blood velocity as a function of location within the lumen. The coordinates of each location are derived from the round trip transit time of the burst of ultrasonic waves from the transducer to the location and return. Therefore, a blood velocity profile can be measured with this instrument. In addition, ultrasonic energy scattered by the walls of the vessel provides the basis for a direct measurement of its lumen diameter. Consequently, the pulsed Doppler flowmeter provides more than sufficient information for an accurate direct measurement of instantaneous volume of flow. Its performance with respect to measurement ambiguities, electronic precalibration, zero drift and scale factor changes is largely equivalent to that of the CW Doppler velocity flowmeter; it is not subject to any inherent shortcomings.

Comparative Features of Flow Telemetry

Comparing the various types of flowmeters, which have been discussed with respect to measurement ambiguities, electronic precalibration, zero stability and scale factor constancy it appears that the CW Doppler velocity flowmeter and the pulsed Doppler velocity profile or volume flowmeter offer the highest levels of performance. The capabilities of these two instruments although generally desirable are especially attractive for reliable chronically implantable telemetry systems.

Considering size and power drain, the principal difference between the flowmeters under discussion is a consequence of the large (e.g., 500 ma) field currents which are needed in the electromagnetic flow transducer. Typical power requirements for electromagnetic flowmeters exceed 500 mw while those of the implantable ultrasonic flowmeters described hereafter are 10–20 mw. As a consequence of their large field current requirements electromagnetic flowmet-

ers must use relatively sizeable battery packs and cannot be implanted unless provision is made for battery recharging through the skin.

The electromagnetic flowmeter cuff must fit rather tightly around a blood vessel in order to provide good electrical contacts between the vessel wall and the three signal carrying electrodes of the cuff. A looser fit is permitted for ultrasonic flowmeter cuffs since direct contact with the vessel walls is unnecessary; however, proper alignment of the transducer(s) must be assured. Because it requires only a single ultrasonic element, which can be mounted in a silastic holder, the pulsed Doppler transducer is perhaps the least traumatic of the various flow transducers.

One of the significant advantages which a telemetry system offers in the collection of physiological data is the opportunity to use unanesthetized, unrestrained, undisturbed animals. By providing the telemetry system with an RF controlled switch or command receiver which connects or disconnects the system (flowmeter) electronics to its power supply upon command from a remote control transmitter, automated data collection can take place at all hours of the day while still maintaining a small average power drain. Ultrasonic flowmeters are well suited to this mode of operation.

A CONTINUOUS WAVE VELOCITY FLOWMETER

A block diagram of a chronically implantable CW Doppler ultrasonic flowmeter which measures blood velocity at the center of the lumen is illustrated in Figure 3.[16–20] The portion of the system within the dashed rectangle is implanted; the remainder is external. In order to reduce its size and power drain as well as improve its reliability, the implantable portion of this system has been designed to use a unique family of low voltage micropower silicon monolithic integrated circuits. This family consists of four chips: an excitor oscillator circuit for energizing the transmitting transducer; an AM receiver circuit for amplifying and mixing the signal from the receiving transducer; an audio amplifier-FM transmitter circuit for transmitting the telemetry signal; and an RF switch or command receiver for disconnecting the flowmeter electronics from its power source during periods when data is not required. The significance of this family of circuits is that it represents the initial application of a custom family of monolithic integrated circuits to a substantial biomedical instrumentation problem. For this reason some of the unique details of the circuit design are discussed in the following paragraphs.

An additional point of interest regarding the flowmeter illustrated in Figure 3 is that it measures only the absolute magnitude of local flow velocity and is not sensitive to direction of flow. A CW system capable of sensing both magnitude and direction of flow velocity is under development in the Integrated Circuits Laboratory of Stanford University at this time.

Exciter Oscillator Circuit

The schematic diagram of the exciter-oscillator circuit is illustrated in Figure 4. This circuit consists of a basic Colpitts oscillator, a buffer-driver amplifier and a class C output stage. The design is optimized in several key respects for both monolithic construction and operation from a single Hg cell power source. For example, the collector current of the oscillator transistor Q_3 is set (equal to that of Q_1) by exploiting the virtually perfect match between two adjacent monolithic transistors Q_1 and Q_2; operating transistors Q_3 and Q_4 at OV collector-base voltage eliminates two discrete (i.e., external to the integrated circuit) bypass capacitors and conserves battery voltage; using direct coupling between Q_6, Q_7 and Q_8 precludes the need for several discrete coupling capacitors; and operating Q_8 with a tuned load permits a collector voltage swing which ideally is twice the supply voltage.

This circuit operates at room temperature over a supply voltage range of 1.0 to 1.35 v with a frequency stability better than 1%; its current drain is 5 ma; it drives the transmitting transducer with a 2 v p-p 6 MHz output voltage at an overall circuit efficiency of 30%; and it uses six discrete chip components— L_1, C_1, C_2, C_4, C_5 and L_2. Figure 5 is a photomicrograph of the monolithic circuit fabricated in a 70 x 90 mil silicon die.

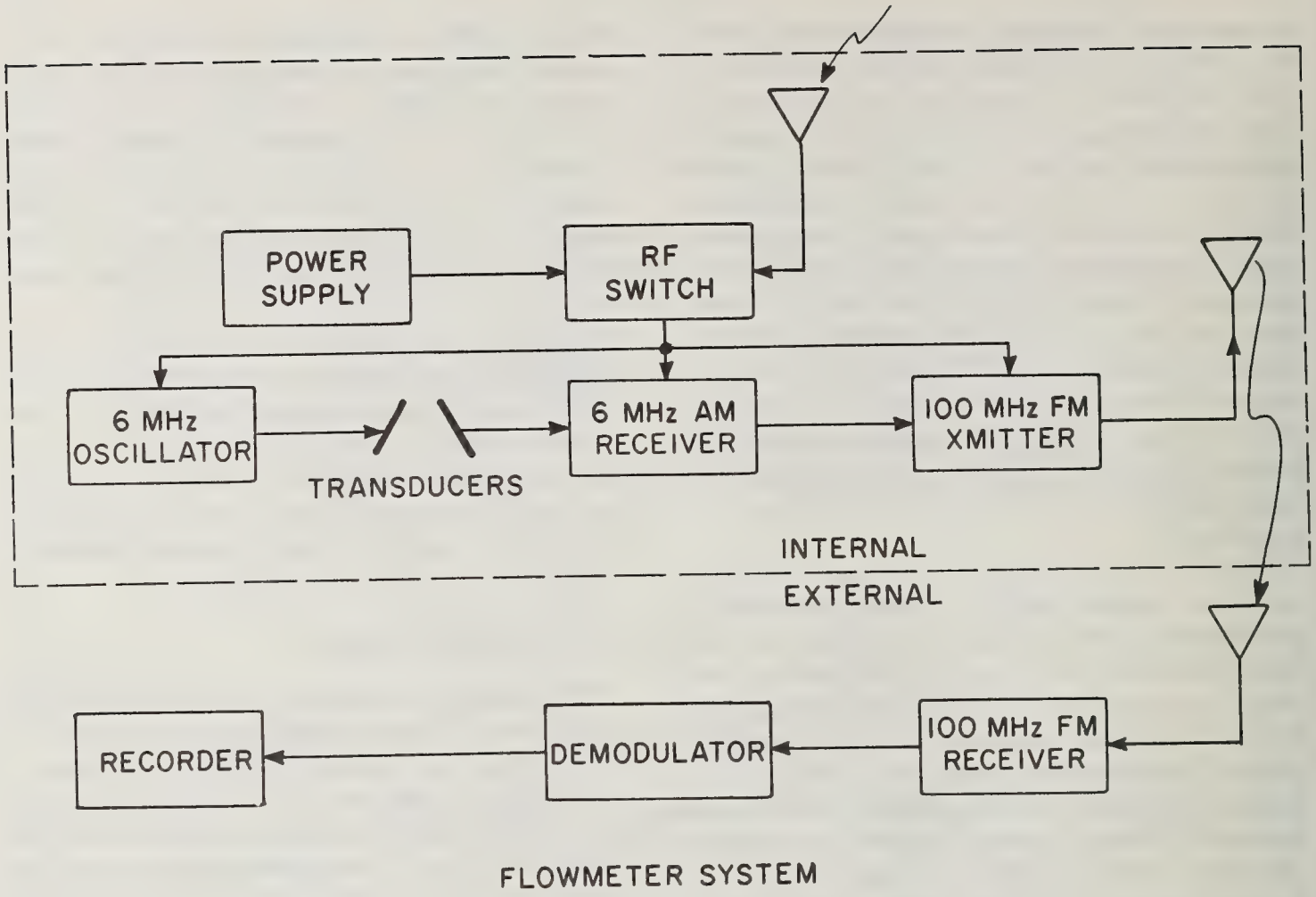


FIGURE 3.—Block diagram of implantable CW Doppler flowmeter.

Low Noise AM Receiver Circuit

A schematic diagram of the low noise AM receiver circuit is illustrated in Figure 6; the circuit consists of an RF amplifier stage (Q_1 and Q_2) a phase splitter (Q_7 and Q_8) and a balanced mixer (Q_3, Q_4, Q_5, Q_6, Q_9, Q_{10} and Q_{11}). Several key features of the design are: close matching of monolithic transistors Q_1 and Q_2 and resistors R_2 and R_3 is exploited in establishing the collector current of Q_2; capacitor C_2 is a small area monolithic element consisting of emitter junction, collector junction and emitter oxide capacitance in parallel; nevertheless, the resonant frequency of L_1 and C_2 is independent of supply voltage since L_1 maintains a dc voltage of 0 v across C_2; Q_3 and Q_5 are operated at 0 v collector-base voltage and Q_4 and Q_6 are actually operated with a very small forward bias on the collection junction in order to conserve supply voltage. Also, in order to provide equal local oscillator amplitude to both halves (Q_9 and Q_{10}) of the balanced mixer from two points at different dc potentials in the phase splitter (Q_8), the monolithic capacitors C_4 and C_5 consist of emitter oxide capacitance only. Finally, the entire concept of using a balanced mixer is utterly unattractive unless low-cost closely-matched monolithic elements are available. The key advantage of a balanced mixer is that the high level carrier is essentially cancelled by the balanced circuit and does not appear at the audio output. Consequently, filter circuitry to remove the carrier becomes unnecessary; the only discrete components needed in the receiver are C_1 and L_1.

This low noise receiver operates at room temperature over a supply voltage range of 1.0 to 1.35 v. Its current drain is 1.0 ma; its sensitivity is 1.0 μv and the conversion gain is

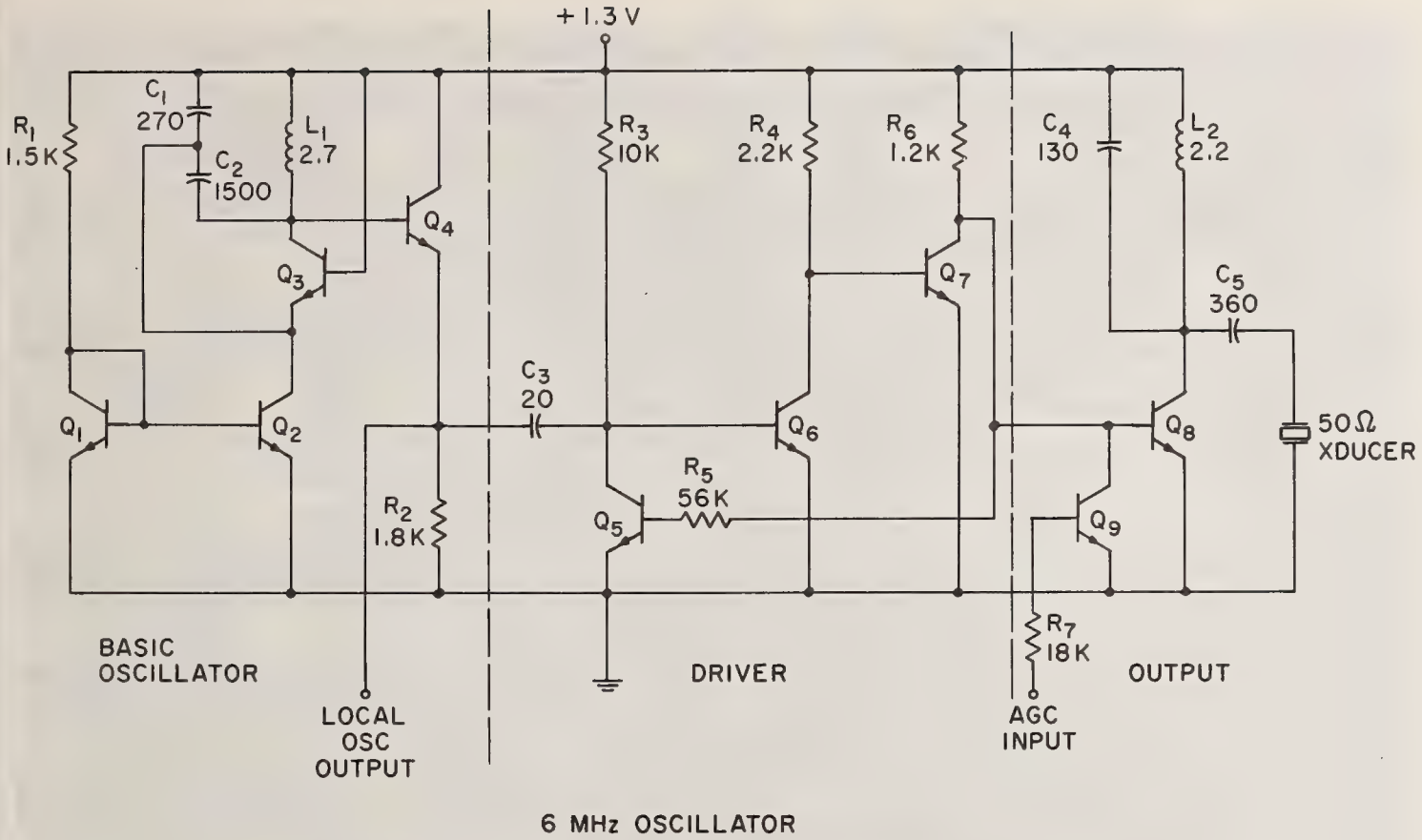


FIGURE 4.—Schematic diagram of CW exciter-oscillator circuit.

40 dB. Figure 7 is a photomicrograph of the 70 x 90 mil receiver chip.

Audio Amplifier-FM Transmitter Circuit

The schematic diagram of the audio amplifier-FM transmitter circuit is illustrated in Figure 8. The active transistors in the audio amplifier are Q_3, Q_4 and Q_8; the active transistor in the transmitter is Q_9. The audio amplifier design makes very free use of the unique features of monolithic technology. The large open loop gain ($\sim$600) of Q_3, Q_4 and Q_8 is achieved by using high dynamic impedance monolithic lateral PNP transistors (Q_5 and Q_6) as load resistors operating at very low dc voltage drops compared with equivalent conventional resistors. The "push-pull" connection of Q_3, Q_4, Q_5 and Q_6 results in no loss of gain in the transition from a differential first stage to a single ended second stage. D_1 and D_2 are low forward voltage Al-Si Schottky diodes which serve to establish a suitable collector voltage for Q_2 while conserving supply voltage as much as possible. To avoid oscillation in the audio amplifier it is internally compensated by C_2 which as a Miller capacitance is effectively multiplied by the voltage gain of the second audio stage. Overall negative feedback (R_2) is used to set the closed loop gain of the amplifier. Symmetrical limiting is provided by including Schottky diodes D_3 and D_4 in the feedback path. The transmitter is a Colpitts oscillator in the common collector configuration (Q_9) to minimize monolithic parasitic effects at 100 MHz; D_5 is the frequency modulating capacitance.

The audio amplifier-transmitter circuit functions at supply voltages as low as 1.0 v. The amplifier gain is 35 dB, the bandwidth 20 kHz and the effective output voltage swing 0.8 v p-p. The transmitter frequency range is 88–108 MHz. The total current drain of both circuits is 1.5 mA. Figure 9 is a photomicrograph of the 70 x 90 mil audio-amplifier-FM transmitter chip; in addition to it, three discrete components are used to implement the circuit.

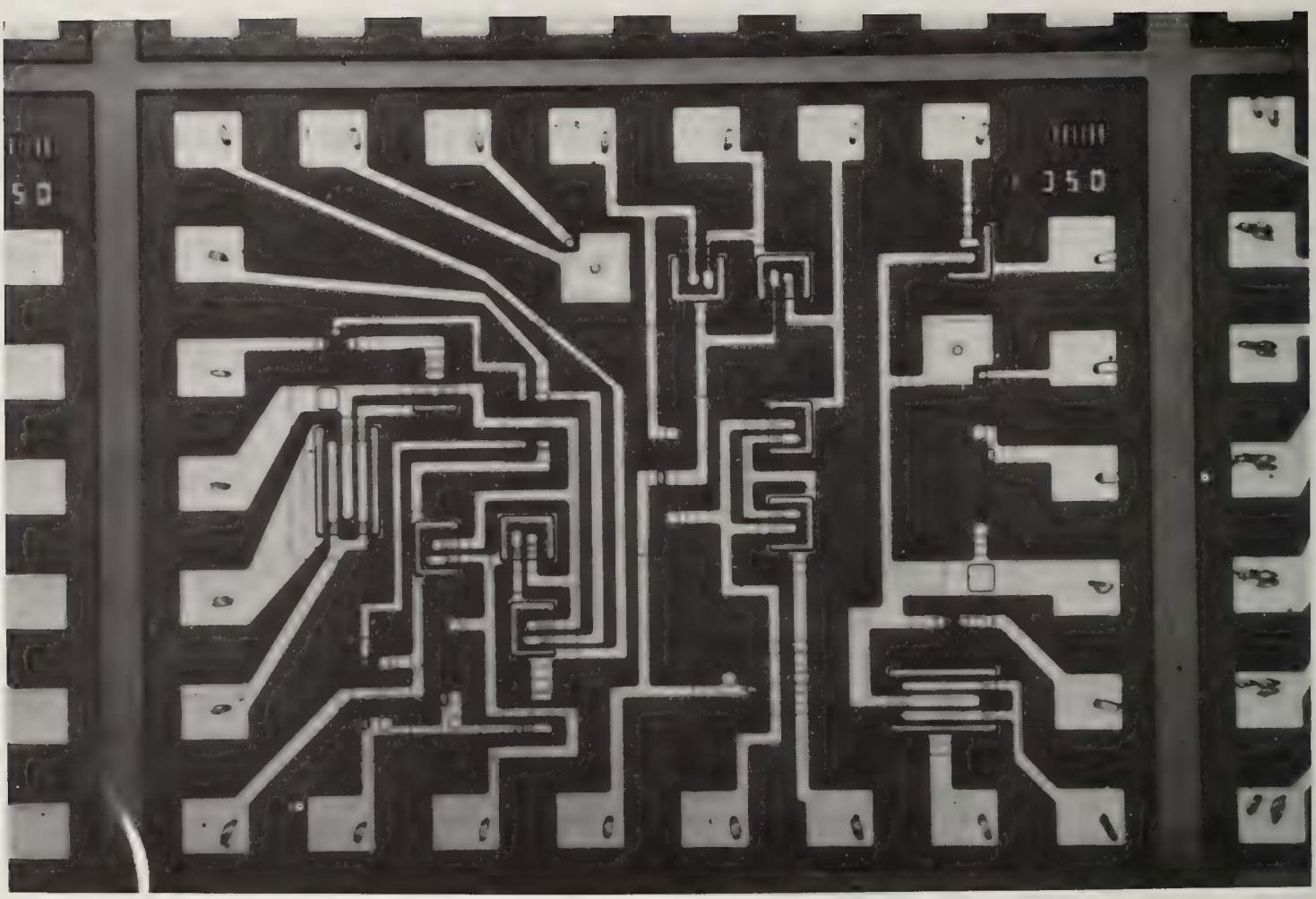


FIGURE 5.—Photomicrograph of exciter-oscillator chip before die scribing and wire bonding.

Command Receiver Circuit

The final circuit used in the implantable system is the command receiver. Its schematic diagram is illustrated in Figure 10. This receiver is a tuned radio frequency (TRF) receiver which operates on a single radio frequency. Consequently, there is no need for a local oscillator or an IF amplifier both of which would require discrete inductors and capacitors. Frequency selectivity is accomplished largely by means of a resonant ferrite rod antenna, although a discrete ceramic filter element between the RF and detector gain cells can be used to improve selectivity. Essentially, the receiver consists of three stages: an RF amplifier gain cell, an AM detector gain cell and an audio amplifier gain cell. The function of each cell is determined by the collector load resistances and coupling capacitances associated with it. All resistors are high value (125 KΩ — 1.5MΩ) base pinch resistors; diode biasing is used to conserve power and avoid excessively high value resistors. The receiver design greatly exploits the constant temperature environment within an animal in its use of these later two techniques.

The most remarkable feature of the command receiver is its ultra low power drain of 13 μW. This feature is essential since the receiver must operate continuously. Because receiver power drain varies monotonically with radio frequency, a relatively low radio frequency (500 kHz) is used. The modulation frequency is 3 kHz; this permits a further reduction in power drain since much of the receiver gain can be achieved at audio rather than radio frequencies. The overall conversion gain is 70 dB and the sensitivity is 100 μV. The receiver drives a

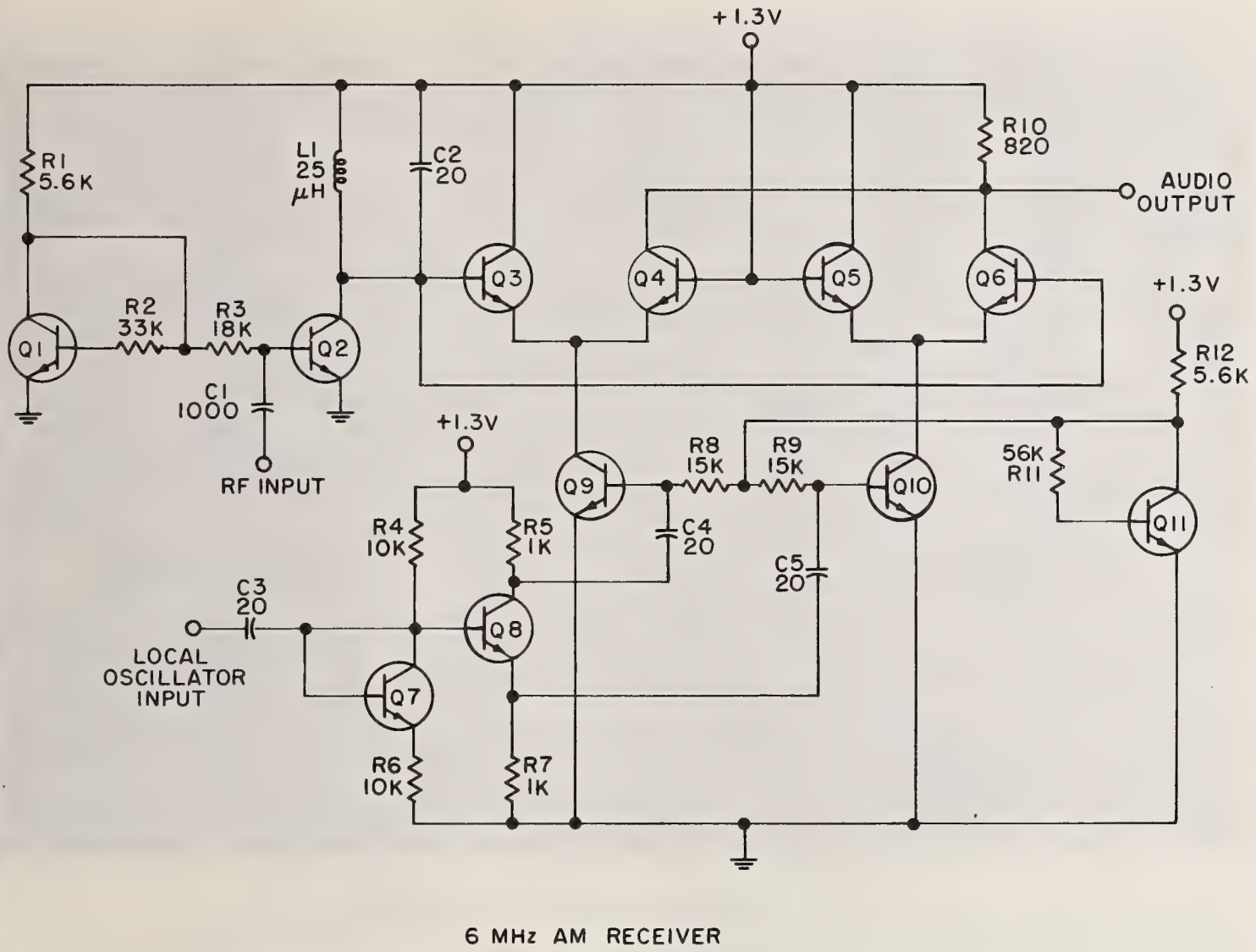


FIGURE 6.—Schematic diagram of low noise AM receiver circuit.

transistor power switch which provides a connection between the flowmeter electronics and its battery power source only during those intervals when a command signal is present. Figure 11 illustrates a photomicrograph of the command receiver chip.

A PULSED VELOCITY PROFILE OR VOLUME FLOWMETER

A simplified block diagram of a chronically implantable pulsed Doppler ultrasonic flowmeter which measures blood velocity profile and lumen diameter and hence instantaneous volume of blood flow is illustrated in Figure 12.[21,22,23] The portion of the system within the upper dashed rectangle is implanted; the lower rectangle contains a block diagram of one channel of an eight channel external system. As with the CW flowmeter, the pulsed system is designed to capitalize fully on the size, power drain and reliability advantages offered by monolithic integrated circuits. Because of the complexity of this system, it is virtually a necessity to implement the implantable portion with integrated circuits specifically designed for the purpose.

As diagrammed in Figure 12, a novel two-oscillator scheme is used in this particular Doppler system. The 6 MHz ultrasonic oscillator which excites the transducer is synchronized to the 20 kHz oscillator immediately before each transmitted burst of ultrasonic waves. This ensures the necessary phase coherence of

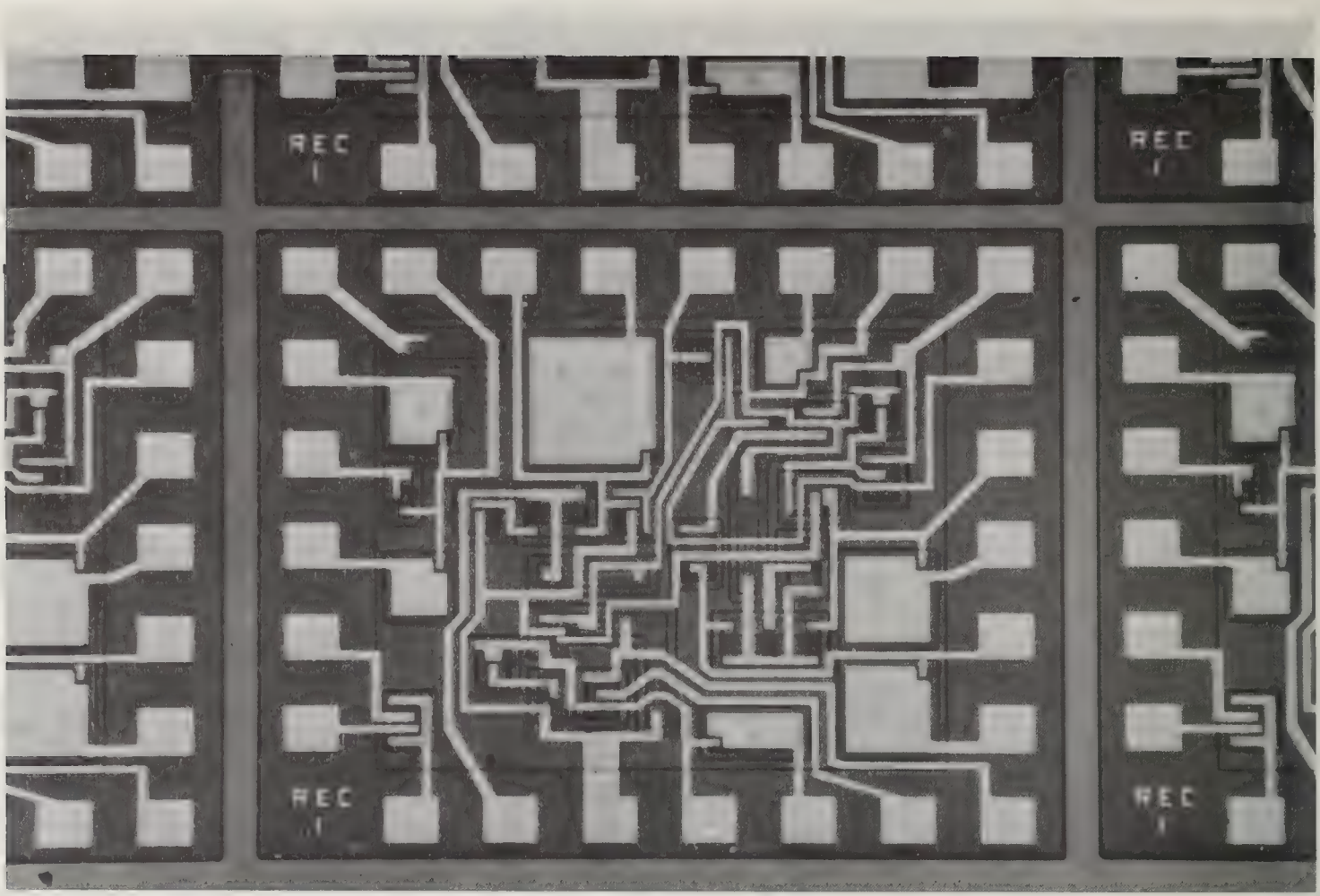


FIGURE 7.—Photomicrograph of AM receiver chip.

the bursts and it is simpler and less power-consuming than the normal technique of deriving the pulse repetition frequency from the ultrasonic frequency by a series of binary dividers. The transducer emits a 1 μsec burst of sound, then acts as a receiver for the following 49 μsec. The received signal is amplified and phase-detected against the 6 MHz oscillator, with the resulting video signal of 0.5 MHz bandwidth telemetered along with a timing pulse. In the external electronics, multiple range gates with different range settings are used simultaneously to give the instantaneous velocity profile across the vessel.

In the integrated circuit design for this system, emphasis is on low voltage operation and low current drain. To minimize the number of discrete components, two Hg cells are used to give $\pm$ 1.3 V supply voltages and thus provide three ac ground points in the circuits. The necessary acoustic power is minimized (about 25 mW peak, 0.5 mW average) by transducer and RF amplifier design; the use of a command receiver enables the flowmeter electronics to be switched on only when data is being collected. Due to the need to measure flow velocity at the near wall of the vessel, transducer ringing time following a transmit pulse and receiver overload recovery time are minimized.

An additional point of interest regarding the flowmeter illustrated in Figure 12 is that it measures the absolute magnitude of the flow velocity profile and is not sensitive to direction of flow. A pulsed system capable of sensing both magnitude and direction of flow profile is now under development in the Integrated Circuits Laboratory of Stanford University.

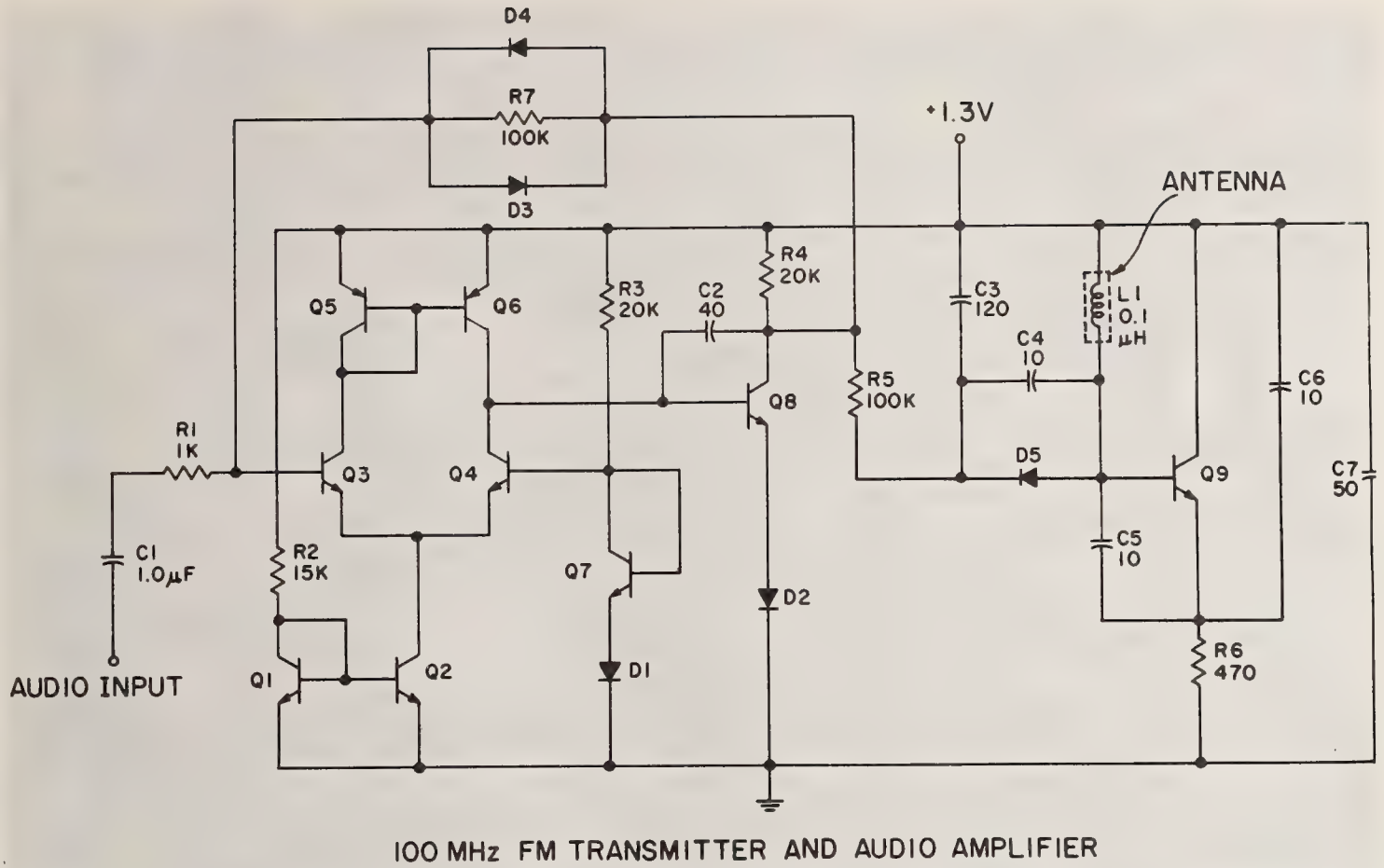


FIGURE 8.—Schematic diagram of audio amplifier FM transmitter circuit.

PERFORMANCE

The instantaneous blood flow velocity in the ascending aorta of a post-operative heart transplant dog is illustrated in Figure 13. The signal was telemetered with a totally implanted CW velocity flowmeter. The transducer was designed as described in Figure 2 to respond to blood velocity at the center of the lumen. Referring to (4), no calibration was necessary since the frequency (f_i) and the sonic angle of attack (θ) were fixed in the design. An accurate flow zero was obtained by setting f_d equal to zero. Drift in the scale factor was negligible since the excitation frequency (f_i) was stable to within $\pm 1\%$.

Figure 14 illustrates the blood flow velocity waveform at the center of the lumen and an eight channel display of the instantaneous blood velocity profile in the descending aorta of an anesthetized dog as recorded with a pulsed Doppler flowmeter.

Although initial tests of the two implantable flowmeters described in this paper have been reasonably encouraging, a substantial effort by a variety of users is needed in order to fully develop the potential of these new instruments.

SUMMARY

Accurate measurement of pulsatile blood flow can be achieved on a chronic basis in research animals through the use of implantable ultrasonic flowmeters. The use of a CW flowmeter to measure flow velocity at a particular location and a pulsed flowmeter to measure velocity profile or volume flow is particularly attractive. Both instruments can be electronically precalibrated and exhibit virtually no baseline or scale factor changes during chronic experiments. Silicon integrated circuits offer significant advantages in reduced size and power drain as well as improved reliability in these instruments. Additional effort is needed to evaluate

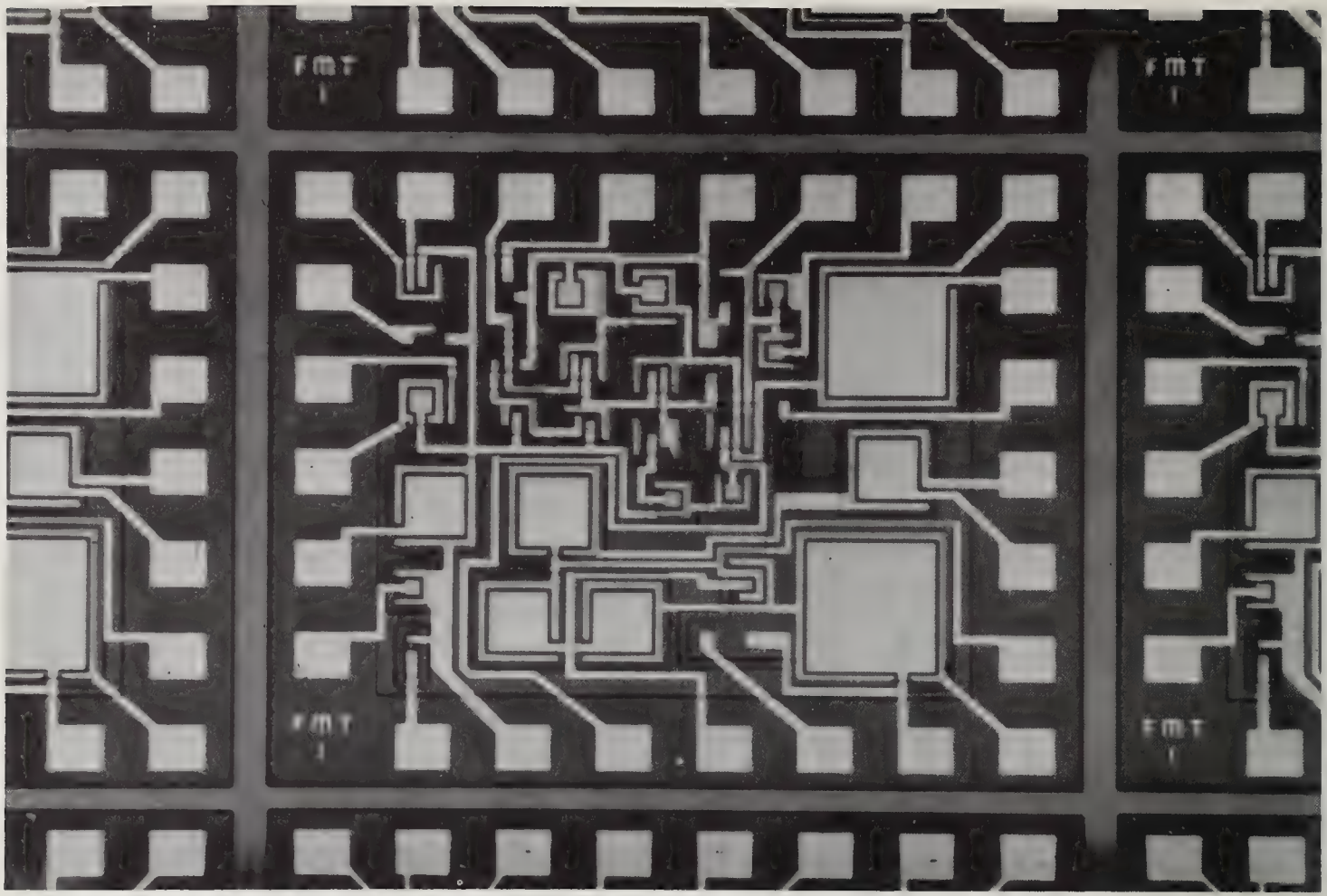


FIGURE 9.—Photomicrograph of audio amplifier-FM transmitter chip.

the instruments and to modify their design to provide a capability for measuring direction as well as magnitude of flow.

REFERENCES

1. SHERCLIFF, J. A. *The Theory of Electromagnetic Flow Measurement,* Cambridge University Press, 1962.
2. WYATT, D. G. Problems in the measurement of blood flow by magnetic induction. Phys. in Med. Biol., Vol. 5, No. 3, Jan 1961 and No. 4, April 1961.
3. FRYER, T. B. and SANDLER, H. Battery operated electromagnetic flowmeter. Proc. 23rd ACEMB, p. 275, 1970.
4. EDGERTON, R. H. The effect of arterial wall thickness and conductivity on electromagnetic flowmeter readings. *Med. and Biol. Eng.* Pergamon Press, 6:627–636, 1968.
5. WYATT, D. G. Baseline errors in cuff electromagnetic flowmeters. *Med. and Biol. Eng.* Pergamon Press, 4:17–45, 1966.
6. FRANKLIN, D. L., BAKER, D. W., and RUSHMER, R. F. Pulsed ultrasonic transit tune flowmeters. IRE Trans. Biomed. Elec. ME–9: 44–49, 1962.
7. PLASS, K. G. A new ultrasonic flowmeter for intravascular application. IEEE Trans. Biomed. Eng. BME–11:154–156, 1964.
8. GESSNER, U. The performance of the ultrasonic flowmeter in complex velocity profiles. IEE Trans. Biomed. Eng. BME–16: 139–142, 1969.
9. FRANKLIN, D. L., KEMPER, W. S. and PIERSON, K. E. Frequency modulated ultrasound transit time technique for measurement of fluid velocity. Preliminary Report, Proc. 8th ICMBE, pp 10–12, 1969.
10. KEMPER, W. S., FRANKLIN, D. L. and VATNER, S. F. Practical implementation of the FM ultrasound flowmeter. Proc. 24th ACEMB, p. 30.11, 1971.
11. RADER, R. D. Cardiovascular telemetry implants. Telemetry J. Vol. 6:15–20, 1971.
12. FRANKLIN, D. L., et al. Blood flow measured by Doppler frequency shift of back-scattered ultrasound. Science 134:564–565, 1961.
13. FRANKLIN, D. L., et al. Technique for radio telemetry of blood-flow velocity from unrestrained animals. Am. J. Med. Elec. 5:24–28, 1966.
14. BRODY, W. R. Theoretical analysis of the ultrasonic blood flowmeter. Stanford Electronics Laboratories Tech. Report No. 4958–1, Oct. 1971.
15. BRODY, W. R., and MEINDL, J. D. Volume flow meas-

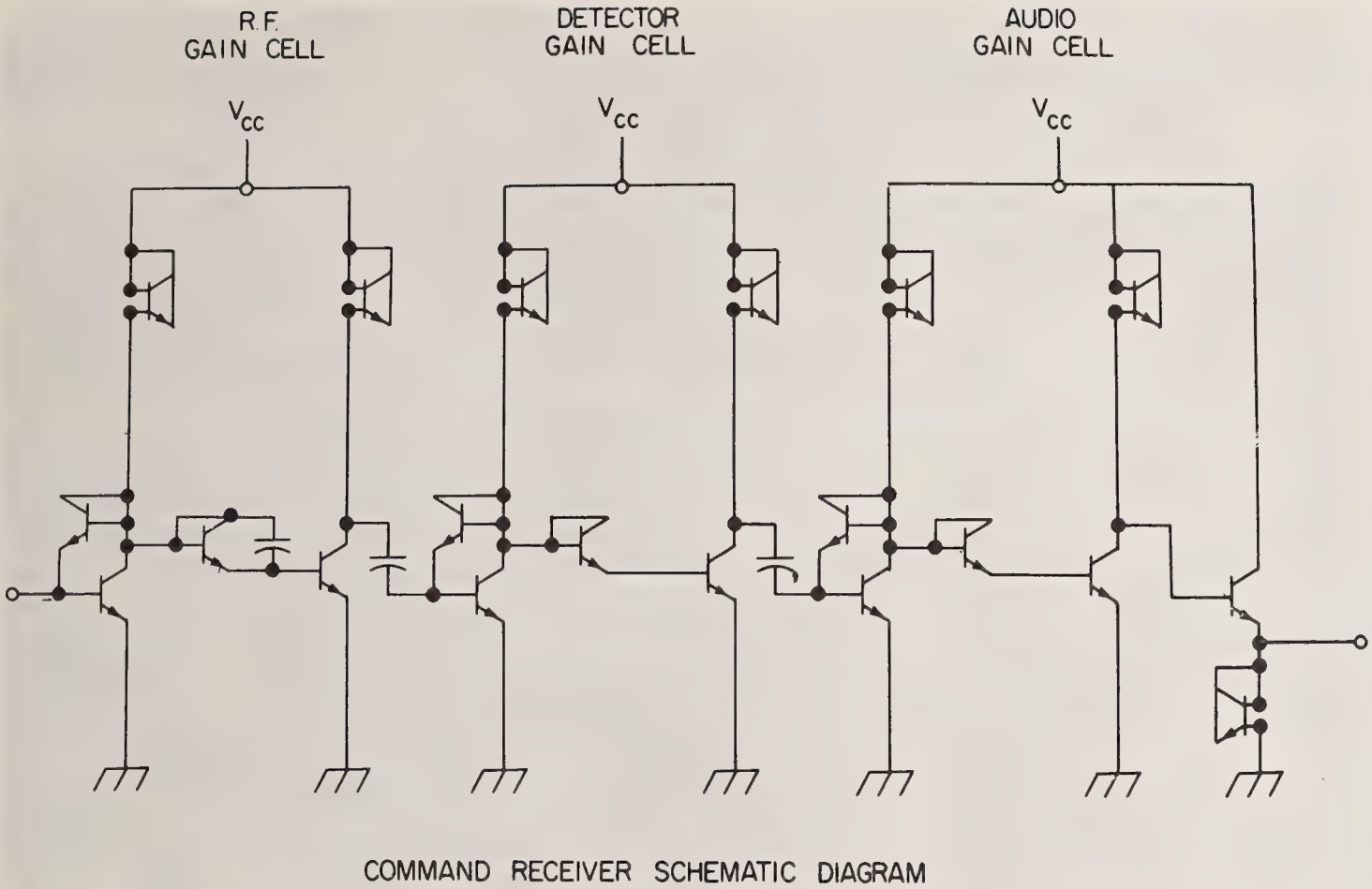


FIGURE 10.—Schematic diagram of command receiver.

urement with the CW Doppler ultrasonic flowmeter. Proc. 24th ACEMB, Oct., 1971.

16. MEINDL, J. D., et al. An implantable ultrasonic blood flowmeter utilizing monolithic integrated circuits. Proc. 8th ICMBE, July, 1969.
17. DIPIETRO, D. M. and MEINDL, J. D. An implantable ultrasonic flowmeter. Proc. 23rd ACEMB, Nov. 1970.
18. DIPIETRO, D. M., MEINDL, J. D., et al. Chronic blood flow measurement in heart transplant dogs. Proc. 24th ACEMB, Nov. 1971.
19. HUDSON, P. H. and MEINDL, J. D. An implantable monolithic command receiver. Proc. 8th ICMBE, July, 1969.
20. MEINDL, J. D. et al. A monolithic command receiver for biomedical telemetry. Proc. 24th ACEMB, Oct., 1971.
21. PERONNEAU, P. A. and LEGER, F. Doppler ultrasonic pulsed blood flowmeter. Proc. 8th ICMBE, July, 1969.
22. BAKER, D. W. Pulsed ultrasonic Doppler blood flow sensing. IEEE Trans. on Sonics and Ultrasonics SU–17:170–185, 1970.
23. GILL, R. W., MEINDL, J. D., and HAASE, W. C. An implantable pulsed Doppler ultrasonic flowmeter. Proc. 24th ACEMB, Nov. 1971.

DISCUSSION

CHAIRMAN FRANKLIN: Are there questions?

P. SOMANI, Abbott Laboratories, North Chicago: I'd like to ask you how you can work the velocity into actual flow. You have not explained, and I am not very familiar with it.

DR. MEINDL: Let's discuss the CW flowmeter first. We use a zero crossing detector to convert the frequency input to a voltage output, and the voltage output has a direct proportionality to the velocity at the position where the beam patterns intersect. It's important that the spectrum be very narrow in order to use such a simple demodulation technique. In the past, very broad, or relatively broad, spectrums, have been used. This simple demodulation technique does not work with broad spectrums in the CW flowmeter.

Now, in the pulse flowmeter, we essentially do the same thing. That is, we look at a very small volume, a volume whose diameter is small com-

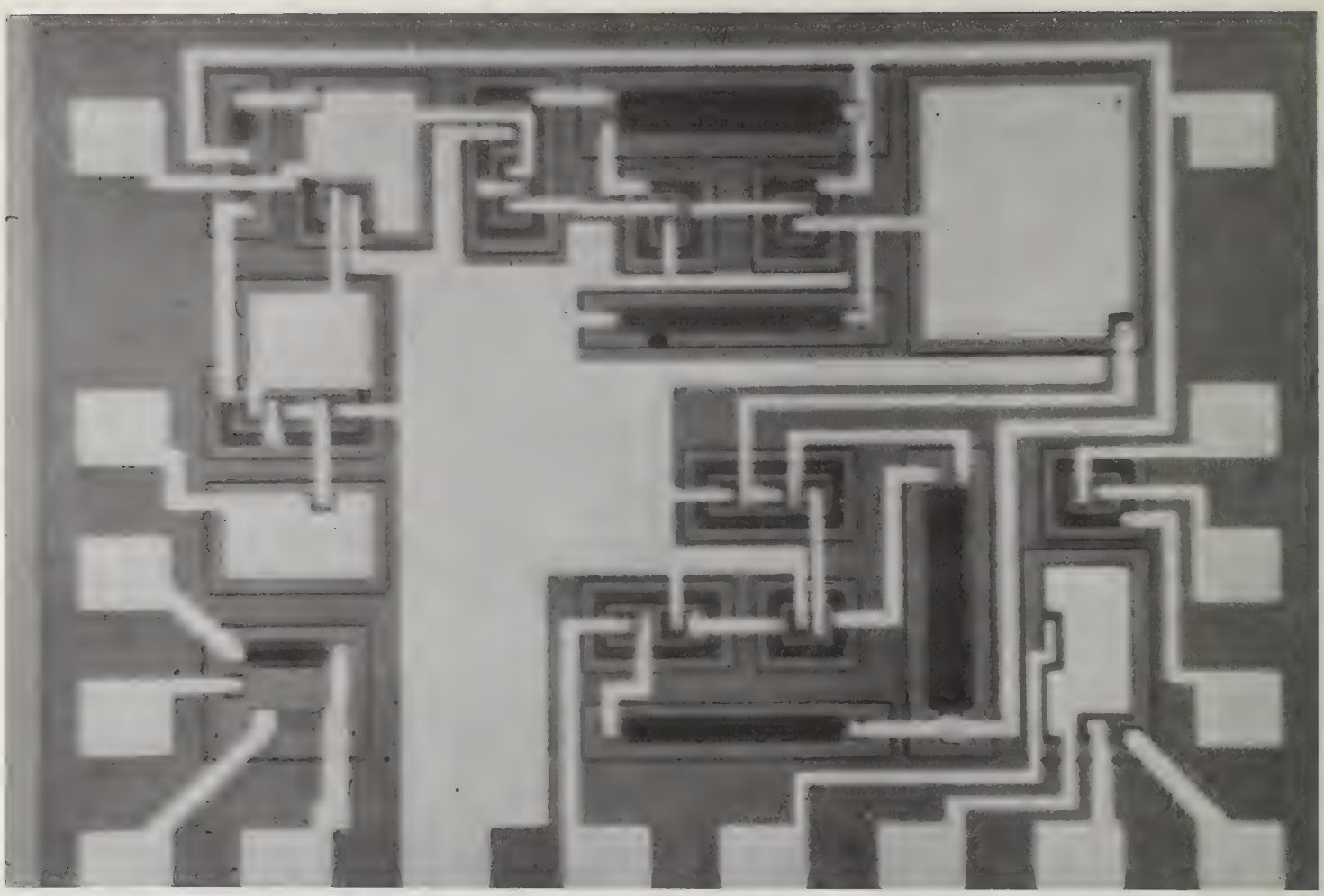
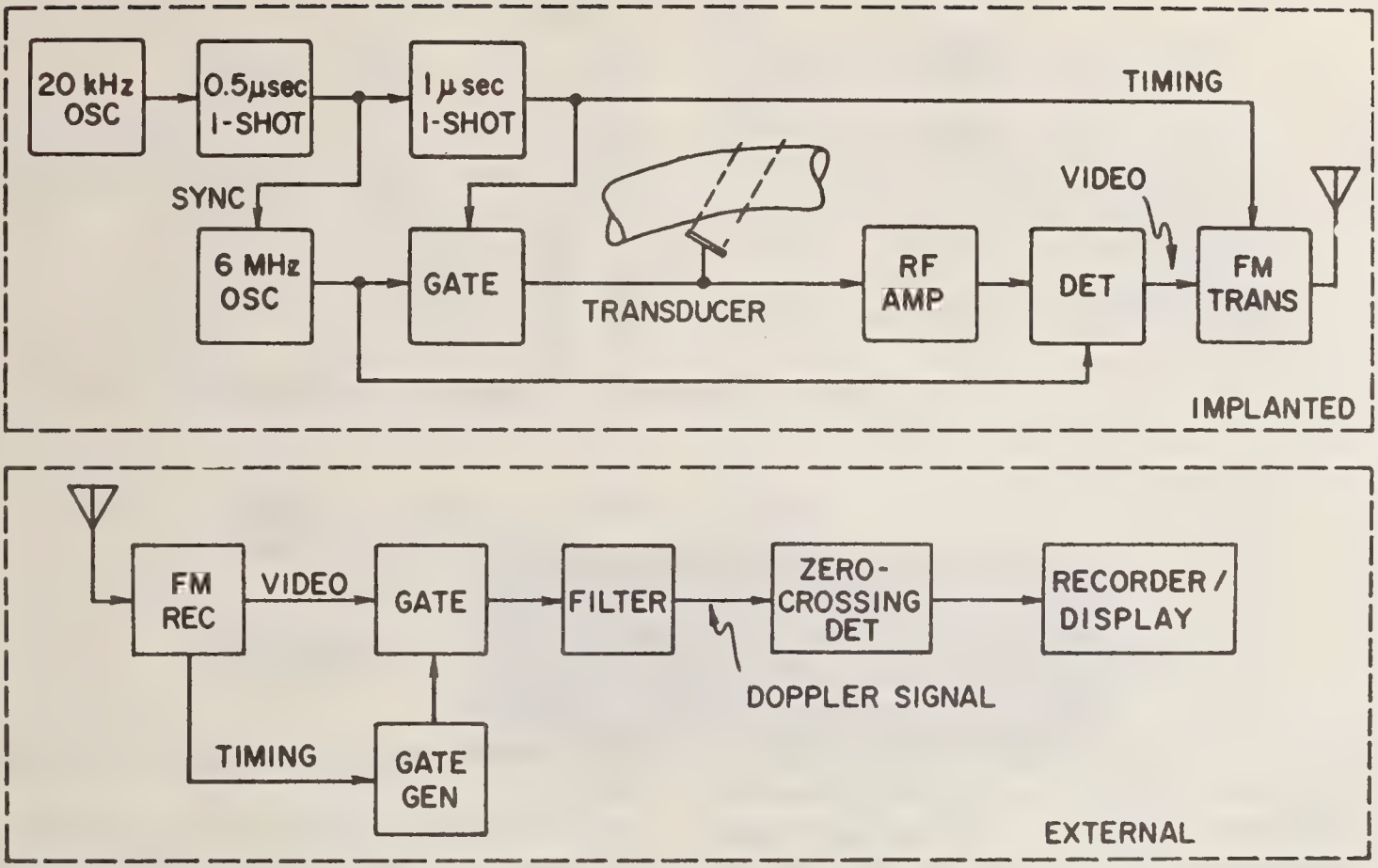


FIGURE 11.—Photomicrograph of command receiver chip.

pared with the lumen diameter, with a given channel of demodulator circuitry, and we essentially make the same kind of frequency to voltage conversion in that channel. You can have a number of channels. We happen to have eight, but you could have twelve or sixteen. We weight the output from each of these channels, depending on the range which corresponds to the radius of that set of targets, then sum that information, and we come up with a single voltage, which is proportional to the volume of flow. This number depends on a direct measurement of the velocity profile and the lumen diameter.

CHAIRMAN: May I take the privilege of the Chairman in disagreeing with you, at least in part, Jim, with regard to the spectrum? Now I can't and I don't think anybody can mathematically justify the fact that a zero crossing detector is usable for converting the wide band frequency information to average velocity. Yet, empirically, we've done this repeatedly only under the conditions where there is a very high signal-to-noise ratio. Under these conditions, we found empirically that there is, indeed, a linear relationship between volume flow and the output of the zero crossing detector.

DR. MEINDL: We don't disagree. I think your point is well taken. We have made (and as yet it is unpublished) a very rigorous analysis of the Doppler flowmeters. I think you've seen a preliminary version of it. The analysis shows very clearly that a linear relationship exists between volume flow and the output of a zero crossing detector if the blood velocity profile is relatively flat or blunt. In addition, if you want to measure volume flow when the blood velocity profile changes rapidly with position throughout the lumen the analysis shows what conditions are necessary to achieve that. I could describe these conditions, although it's a more



IMPLANTABLE PULSED ULTRASOMIC FLOWMETER-BLOCK DIAGRAM

FIGURE 12.—Block diagram of implantable pulsed Doppler flowmeter.

mathematical set of conditions than you can handle easily in a verbal discussion.

Essentially, you have to take the integral of frequency times the spectral power density from plus to minus infinity. In other words, you take the first moment of the spectral power density and that gives you the average Doppler frequency. Now in order for this to give you the average Doppler frequency, you have to assume that you insonify the cross section of the vessel with the ultrasonic energy. In other words, it's essentially the same condition you have in the electromagnetic flowmeter; that is, the magnetic field has to be uniform. If you insonify the entire cross-section of the vessel in the CW flowmeter and then do this calculation that I just described (instead of doing a simple zero crossing demodulating process) you can measure the average velocity over that cross section. If you know the average velocity, then assuming you know the lumen diameter, you can come up with a volume flow.

Now, my estimation of the best technique (and I'm not saying at all that you can't get useful data on volume flow with the CW flowmeter) is to use the CW flowmeter to measure velocity in a very small volume. The best way to use the pulse flowmeter is to measure a velocity profile and a lumen diameter, and this gives you the basis for measurement of volume flow. I might point out that if you use the instruments this way, you can precalibrate them. The user does not have to calibrate them; the calibration is stable; and the baseline is stable.

CHAIRMAN: I have no objection to what you say at all. I think we agree with your point. But really the Pulse Doppler is the only volume flowmeter available at the moment. I guess the point I was getting to was perhaps the lesser one than you referred to. The point is that

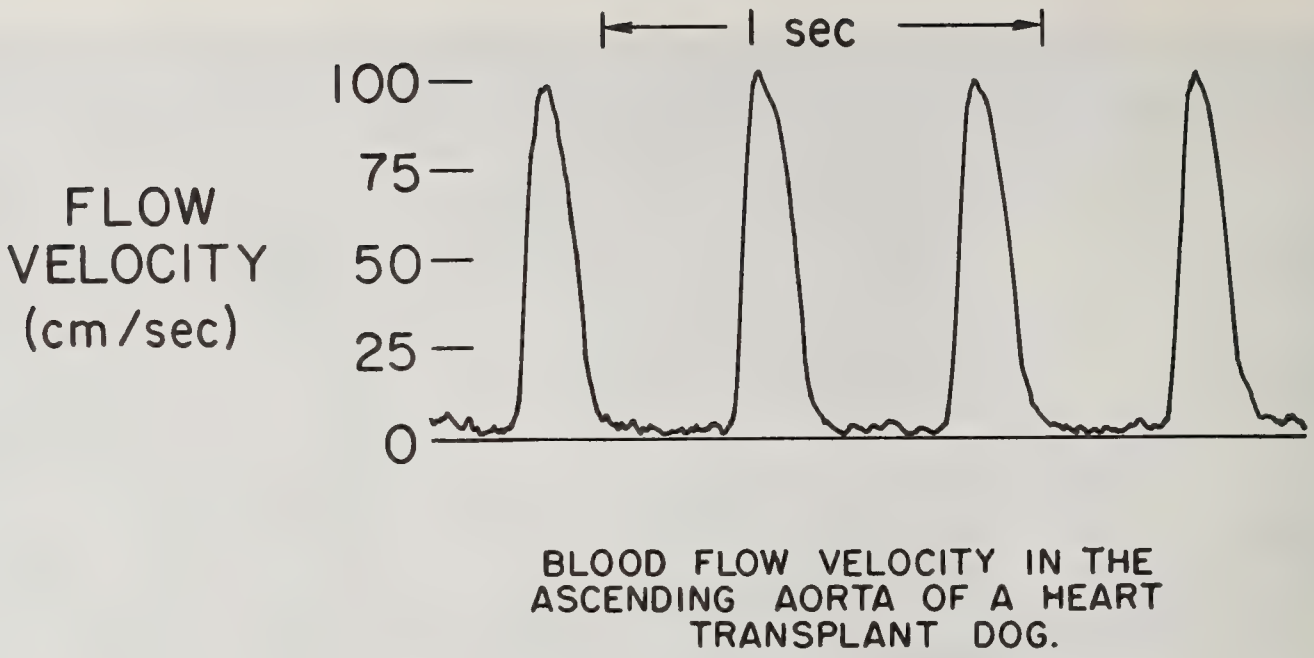


FIGURE 13.—Blood flow velocity waveform recorded with the CW Doppler flowmeter.

we've used it for a number of years, we zero-cross detect with a zero-crossing detector. The signal-to-noise ratio was high and we illuminate most of the vessel. So with these assumptions (and in fact you explain why), I merely said empirically we found this to be so. Is that fair? I objected to your saying that you can't use a zero detector to derive volume flow. We differ in the term "derive," I guess.

DR. MEINDL: I think what you have stated is fair. Under certain conditions such as a blunt velocity profile and insonification of the cross-section of the lumen, the CW Doppler flowmeter can provide a measurement of volume flow; under other conditions it may not.

CHAIRMAN: I think we agree and I've taken up far too much of your time. I'm sorry. There's a question there.

DR. LANDSCOOL, Seattle: I would like to point out that the Pulse Doppler is extremely versatile not only as a volume flowmeter, but if used with a scanning arm, the percutaneous sonar arteriographic picture of an artery can be obtained, for example, the carotids or the femorals. We've also used it to determine patency and to make pictures of saphenous vein bypass graphs. This is also an additional application that you haven't mentioned, but an extremely important one, in addition to quantitative flow. Thank you.

M. E. SIKORSKI, Georgia Tech, Atlanta, Ga.: I'm wondering what accuracy can be obtained as far as flow measurements are concerned. In certain applications, this is quite an important problem, especially in brain surgery.

DR. MEINDL: To date in our work we have not established a reliable estimate of the accuracy of the Doppler ultrasonic flowmeter. My rough estimate is that its accuracy may be limited to $\pm$ 5% of 0.

W. S. TOPHAM, Artificial Heart Test Facility, Salt Lake City: I noticed that in the recording that you showed of the aortic flow wave form, the reversal flow was notably absent. Is this because your flowmeter is not detecting the reversal of flow or is this because of the preparation?

DR. MEINDL: I restricted myself here to talking about nondirectional flowmeters because they are the simplest ones and because we're further along in the work we're doing on them than we are on the bidirectional systems.

DR. TOPHAM: Thank you. I wanted to make sure that that point was understood because the electromagnetic flowmeter seems to not have these disadvantages, and in the comparison, I think it's fair to make that known.

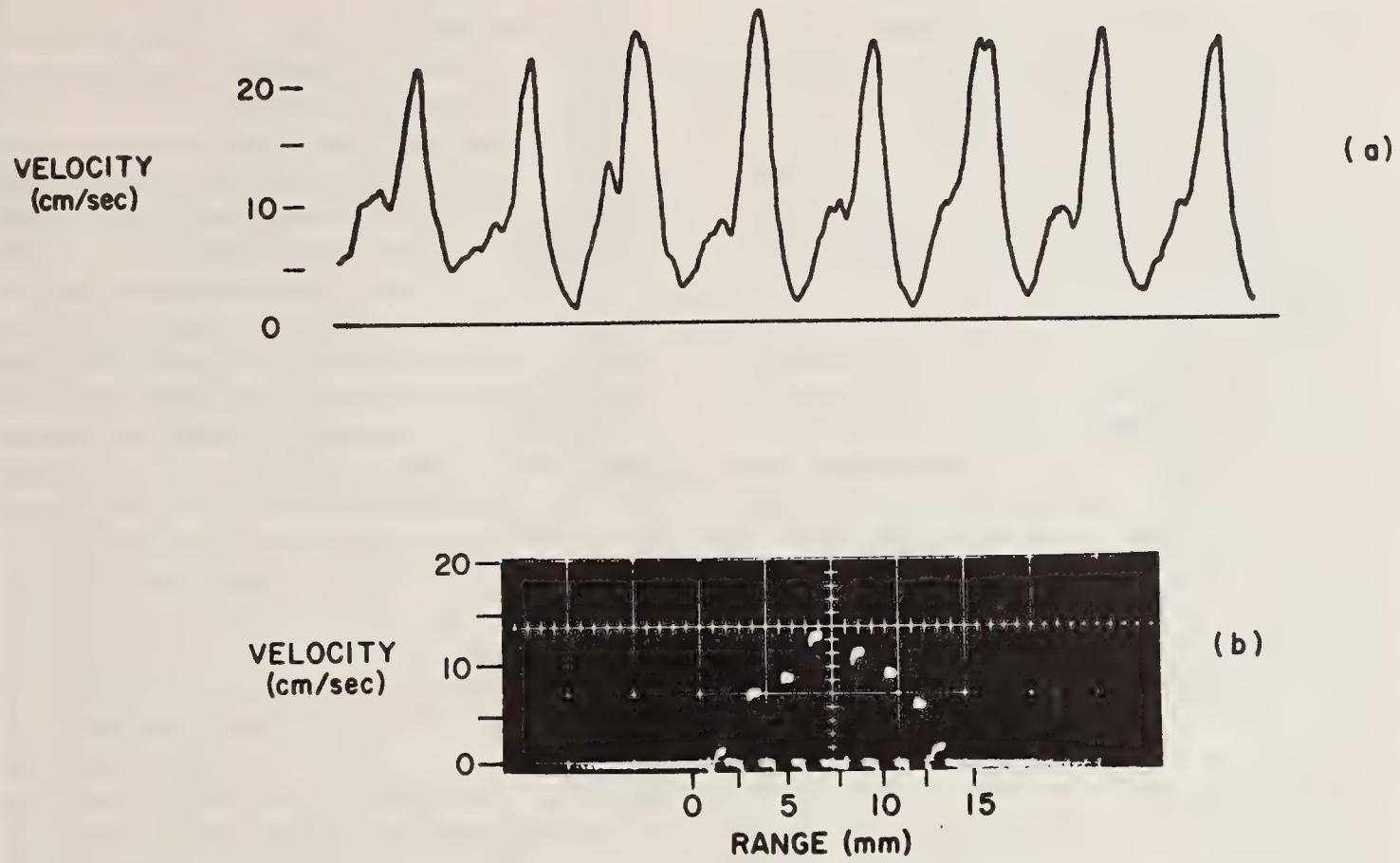


FIGURE 14.—Blood flow velocity waveform and velocity profile recorded with the pulsed Doppler flowmeter.

DR. MEINDL: May I ask a question? You said "disadvantages"; could you define them? I think people would be interested in them.

DR. TOPHAM: One of the criteria that you suggested was that you be able to use precalibrated probes and that you also have a standard electrical zero.

DR. MEINDL: That's correct.

DR. TOPHAM: Well, the electromagnetic flowmeter gives us these same capabilities now without the problem of having to worry about the non-reversal of flow or the reversal of flow; they're precalibrated probes. Admittedly, they're not electronically precalibrated, they have to go through some sort of calibrating procedure. But they're guaranteed within plus or minus 5 per cent accuracy that you stated and electrical zero which I showed in my paper is very good. It's very reliable now in many of the flowmeters that are available.

DR. MEINDL: I would like to make it clear that although the ultrasonic flowmeters I described in my paper are nondirectional, there is no inherent reason why an ultrasonic flowmeter cannot measure bidirectional flow. In fact, we are quite actively engaged in designing bidirectional ultrasonic flowmeters using both the CW and pulsed modes of operation. These systems can be precalibrated.

DR. TOPHAM: I have not seen a bidirectional flowmeter, and I know that's my ignorance. I hear them discussed but I've never seen one.

DR. MEINDL: The reason we're working on it is because there aren't any available.

CHAIRMAN: One moment here, I might make a comment again as Chairman and as having worked with the Doppler flowmeter and also having worked with the electromagnetic flowmeter and with a number of other flowmeters. I'll say without regard to anybody else, I've yet to see a flowmeter that I've convinced is accurate to plus or minus 5 per cent, and it will be some time, I believe, before a manufacturer can stand behind that. I'll be very happy to see it and I'll probably use the thing if I can possibly find one.

QUESTIONER (Unidentified): What do you

use to determine whether it's plus or minus 5 per cent? What is the standard, quite precisely?

CHAIRMAN: I use a graduate and a stop watch, and there is no alternative.

QUESTIONER (Unidentified): A trivial question—in your pulse flowmeter, you show a neat parabolic cross section of flow profile, and I assume that's why Dr. Franklin got his linearity between the empiric data and the device he used. I'd like to ask if you have any built-in assumptions as to what the velocity profile has to be when you'll be working with pulse?

DR. MEINDL: I think the fortunate thing about the pulse flowmeter is that you can be entirely devoid of any preconceptions about what the profile is and it still does the job for you. It will tell you exactly what that profile is.

B. W. SAUER, Clemson University, South Carolina: My question is in reference to the, again, velocity profile. Could we assume that such an instrument could be used to measure the variable velocity profile, say, in turbulent flow?

DR. MEINDL: The answer is simple—no. In the instrument that I use or that I described, you could not calculate volume of flow or velocity in turbulent flow. If you want to do that, it requires the use of additional transducers. It can be done, but it's a more complicated process than the one that I described.

CHAIRMAN: Is it conceivably feasible?

DR. MEINDL: I feel that it's conceivable that you could do it.

CHAIRMAN: So do I. I think that it shows very much promise on that very basis, that it shows promise of providing precisely the thing that you indicated. That is why I agree with Jim that a Pulse Doppler, theoretically at least (there doesn't exist a practical one as yet), shows promise of providing a measure of absolute volume flow. It is the only instrument that does show that promise in a practical sense.

I think it's very clear that from this afternoon's work that while it may be possible to telemeter things, it's difficult to put them into a form II telemeter. The measurement is the major difficulty. We still obviously are having considerable difficulty measuring blood pressure and blood flow. There's no question of it, and so I'm happy to see such good people working in these very areas.

Now then with regard to our visitor from France. He's used a Perouneau pulse flowmeter and he has a very brief comment.

D. Y. LOISANCE, Broussais Hospital, Paris, France: For two years, we have been using a Pulse Doppler flowmeter designed by Dr. Peronneau in Paris. May I have the first slide please? This Doppler flowmeter allows the recording of velocity profile in a vessel. Zero is obtained without occluding the vessel. We can recognize internal diameter of this vessel on the echogram. To assess the volume flow, we assume that the profile recorded on one diameter of the vessel is representative of all of the diameters. And by a semi-annular arranging of a small part of the flow, we can guess the total volume flow. May I have the second slide please?

We use this flowmeter in a lot of demonstrations in dogs and in humans, in acute and in chronic experiments. In an acute experiment, we record profile of velocity in different parts of the aorta. I will speak to you about the readings we get in the portal vein and hepatic artery before and after a side-to-side portacaval shunt. We choose this model because we were expecting a very strange velocity profile. I put velocity transistor on the portal vein in two different locations—one on the distocervical portal vein just on the side of the portacaval shunt and one before the portocaval shunt. An additional probe is placed on the hepatic artery. We can change the outflow resistance of the hepatic circulation by a snare which controls the flow in the hepatic vein. May I have the next slide?

These are aorta regions in normal dogs. You can see a velocity profile in the different parts of the portal vein. This velocity profile is never parabolic and very often, in 50 per cent of our dogs, we get this very strange velocity profile. It seems like we have two different parts in the flow. Here is hepatic artery velocity profile and in the hepatic artery we never get parabolic velocity profile. May I have another slide?

Here again is a normal velocity profile in a normal portal vein in a different part of the portal vein. Here is a velocity profile downstream to the side-to-side portocaval shunt in two different conditions. Here is a suprahepatic vein open and here is one closed. Here we can record the bidirectional flow. In this condition

we always get this negative profile. May I have the next slide?

We also recorded velocity profile just in the side-to-side portocaval shunt in two different planes. Here is a graphic representation of the side-to-side portocaval shunt. Here the portal veins are anterior to the vena cava vein and here is the inferior vena cava. The probe is here. We can record a negative flow in this spot and a positive flow here. If we eliminated the paraventricular vein, we can see velocity profile positive from the portal vein and inferior vena cava. We could not complete the flow through the anastomosis in this situation because of the two different flow patterns in different veins. May I have the next slide?

We also tried to do some study on chronic dogs. If we had not too many problems with arteries, it appears possible to record good velocity profile on portal veins. Here is a velocity profile obtained during surgery. And here it is three weeks after surgery. So velocity is almost double as your lumen diameter is decreased. May I have the last slide please?

When we are doing anatomical studies, we can see that the probes have induced an enormous inflammatory response at the site of the probe. I thank you very much.

PERCUTANEOUS CONDUCTION OF ELECTRICAL SIGNALS: INTERFACE TECHNIQUES AND TISSUE REACTION AT ELECTRODE SITE

J. T. Mortimer and P. H. Peckham*

The use of hard wires for transmission of an electrical stimulus or for information retrieval has advantages over telemetry systems in many experimental procedures. The advantages of hard wiring are: an electrical stimulus may be more precisely controlled, quantification of recorded signal is easier, there are fewer problems with electrical noise, and it is less expensive than most telemetry systems. A major problem that one encounters with this technique is to maintain an "infection-free" interface. The ability to maintain an "infection-free" interface often determines the success of an experiment, particularly in long-term chronic experiments. The term "interface" is used here to designate any mechanical device which is implanted in the skin and through which lead wires pass. The purpose of the interface is to prevent infection and to anchor the lead wire. Subjective evidence indicates that the invasion of bacteria causing infection probably takes place via two mechanisms: 1) Bacteria may be carried into the body if the lead wire is permitted to slide freely in the wound through which it enters the skin; 2) Bacteria may also gain access through the small fissure between the skin and the wire or the interface. Several interfaces (using synthetic fibers and porous carbons) have been designed with these parameters in mind. The implant results indicate that it is possible to maintain wires through the skin for prolonged periods of time utilizing interface techniques which minimize slippage and fissure size. The Caldwell electrode has been found to be the most successful for both information retrieval and stimulation. The electrode has been implanted in muscles with good stability and minimal tissue reaction in passive implants. Utilizing the electrode as a stimulating electrode, histological studies indicate that the tissue reaction is not statistically different from the passive implants for stimulus parameters in the physiological range (100 μsec, 50 Hz, amplitude < 15 mA).

INTRODUCTION**

There are two basic ways to transfer electrical signals from one side of the skin to the other, telemetry and percutaneous wiring. To rate categorically one technique over the other is inappropriate since neither approach is without certain disadvantages. There are, however, certain precautions one can take to minimize these disadvantages. With these precautions in mind, one can choose the method best suited for a particular experimental preparation. Briefly, a disadvantage of telemetry is that relative motion between the receiver and transmitter antenna may introduce an undesirable error in the electrical signal. Also, calibration and maintenance of calibration can be rather difficult. Other than the possibility of restricting the animal's movements, the primary problem with percutaneous signal transmission is maintenance of an "infection-free" interface. The following text is devoted to methods which minimize the problems of an "infection-free" interface. In addition, material is presented which covers aspects of an electrode system suitable for chronic recording and stimulation. Methods of minimizing the disadvantages associated with telemetry are discussed elsewhere.

The following text is separated into three sections. The first is concerned with methods which can be used to minimize the problems of maintaining an "infection-free" interface. The second and third sections cover test results with an electrode configuration found to be suitable for long-term use and methods of connecting this electrode to a lead wire respectively. The material in the latter two sections is applicable to systems utilizing either telemetry or percutaneous wires.

THE INTERFACE

A major problem encountered with percutaneous wiring is the maintenance of an "infection-free" interface. The term "interface" is

* Case Western Reserve University, Cleveland, Ohio.

** This research was supported by the Social and Rehabilitation Services Grant Number 23-P-55207/5-02.

used here to designate any mechanical device which is implanted in the skin and through which lead wires pass. The purpose of the interface is to prevent infection and to anchor the lead wires.

Subjective evidence, gathered in this laboratory over the past years, indicates that the invasion of bacteria, causing infection, takes place via two mechanisms:

1. Bacteria may be carried into the body if the lead wire is permitted to slide freely in the wound through which it enters the skin;
2. Bacteria may also gain access through the small fissure between the skin and the wire of the interface.

Therefore, bacterial invasion may be minimized by eliminating wire slippage and keeping the skin-interface fissure to the smallest possible size.

Anchoring the wire (with its insulating covering) to the interface eliminates wire slippage in most cases. The major problem then lies in minimizing interface movement or fissure opening between the skin and the interface.

To date, most interfaces have a dumbbell shape and are formed from polymer materials with smooth surfaces such as teflon and silicon rubber. The wire or wires are embedded in the center portion of the interface and the skin is closed in such a way to place one end of the dumbbell below the skin and the other on the outer surface of the skin. Two major drawbacks to this configuration are:

1. Fissure size is not minimized but made larger by placing the wires inside the center section of the dumbbell. The minimum diameter of the fissure corresponds to the diameter of the stem or shank of the dumbbell.
2. The use of solid materials such as teflon and silicon rubber prohibit attachment of tissue to their surfaces. This arrangement then allows a constant mechanical irritation of the tissues around the interface resulting in cellular destruction and chronic inflammation.

Ideally, one would like to use a material to which body tissues adhere. To date, there is no known inanimate material to which the living cells of the body attach themselves. A substitute for the ideal is a biocompatible material with a structure which allows tissues to grow into it or through it. The ingrowth of connective tissue substitutes for the adherence to act in anchoring the interface. Materials with these characteristics are fabrics and porous composites. A number of materials falling in these categories have been tested by implantation and are found to be biocompatible. Fibers woven into meshes and velours that have been extensively tested are dacron, nylon, and teflon. Porous composites tested or under test are ceramics [1] and carbons.[2] Both show promising results. Histological examination of the implant site has shown a significantly milder foreign body reaction when porous materials are used than when similar impervious materials are used.[1] At the present, about half of the composite materials have been tested as percutaneous devices with the remainder under consideration.

The use of a carbon material as an interface suggests that this conducting interface might also act as an indifferent or reference electrode. However, the results obtained by Gibbons, Peckham, and Martin [3] indicate that severe degradation can occur under anodic conditions. Therefore, it is not recommended that a carbon interface be used as an anode electrode.

In a modification of the teflon dumbbell shape, Miller [4] has attached a synthetic fiber velour to the small diameter section. Epithelium reportedly grew into the velour, forming a satisfactory interface.

An alternate approach that has been extensively utilized by the authors was described by Gertler et al.[5] The sketch shown in Figure 1 depicts a cut-away of an implanted interface. In Figure 2 is shown the actual interface prior to implant with the lead wires attached to it. This technique, unlike the dumbbell configuration, bonds the interface to the electrode lead exterior to the epidermis. The largest diameter of any hole through the skin then becomes the diameter of the insulated wire used for signal transmission.

Slippage of the lead wire through the skin into the subcutaneous space is minimized by placing the silicone rubber ridge (to which the lead wires are attached) next to the skin. Out-

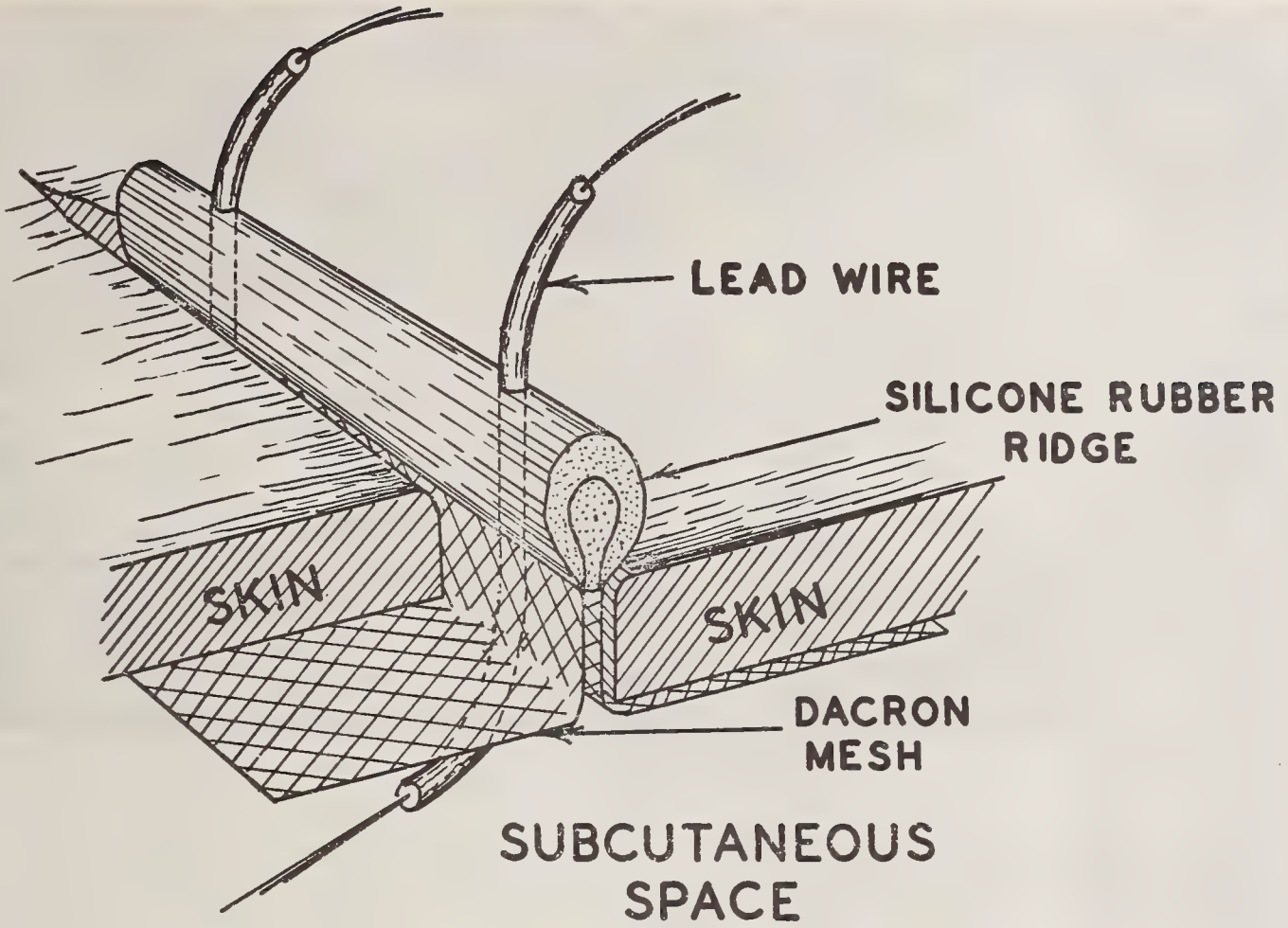


FIGURE 1.—Cut-away drawing of implanted dacron mesh interface.

ward movement of the wires is restrained by the Dacron mesh through which connective tissue of the skin and the subcutaneous space rapidly grow. The ingrowth of tissue acts as a mechanical lock on the interface, substituting for the ideal case of tissue attachment to the interface.

The procedure for fabrication of the interface is slightly modified from that given by Gertler et al.[5] and is as follows:

1. Dacron mesh is cut into a strip measuring 1.5 by 6 cm. (These dimensions are suitable for a two lead interface with 2 mm O.D. leads.)
2. The Dacron strip is folded in the middle, forming a 1.5 by 3 cm double strip. Metal rods with the same diameters as the lead wires are placed through the folded mesh in the same position the lead wires are to occupy. Experimentally it has been found that the wires should be at least 6 mm apart and no closer to the edge of the strip than 4 mm. (Wires placed too closely together or too near the edge of the strip result in poor anchoring.)
3. Silicone rubber (RTV) is then applied to the folded portion of the Dacron and around the metal rods to form the silicone rubber ridge. After the silicone rubber has vulcanized, the metal rods are withdrawn from the interface. At this point the interface appears as shown in Figure 2 and is suitable for autoclave sterilization.

Implantation of the interface is as follows:

1. The implant site is shaved and swabbed with an antiseptic solution;
2. An incision, approximately 2 cm, is made in the skin at the desired site and the subcutaneous tissues are separated to allow placement of the Dacron tabs;
3. The lead wires from the electrode site are

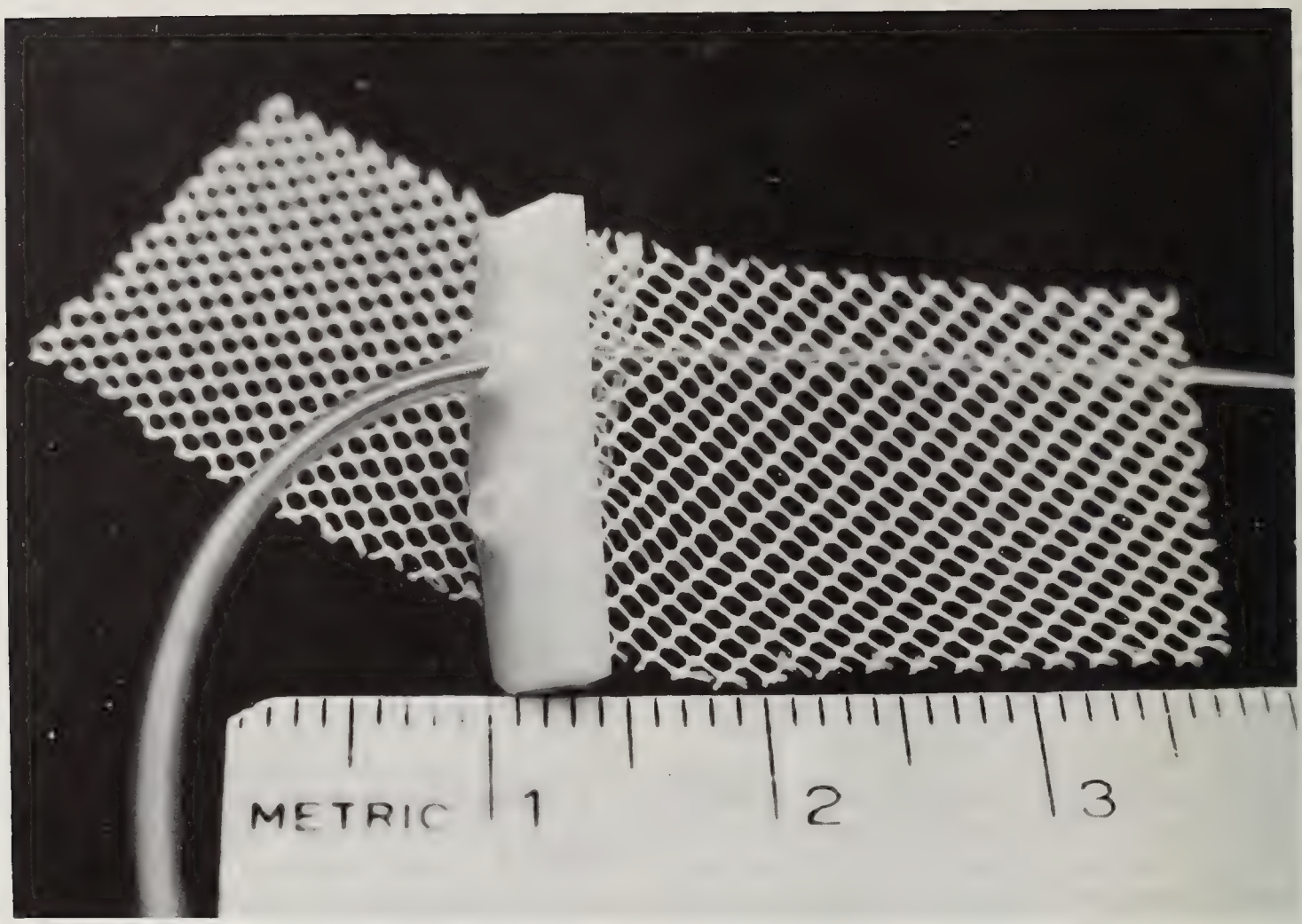


FIGURE 2.—Dacron mesh interface with one or two lead wires in place.

drawn through the incision site and threaded through the holes;

4. Medical Grade A Adhesive (Dow Corning) is applied to the lead wires at the point where the wires are to exit the animal's body. The adhesive acts to bond the lead wire to the silicone rubber ridge. A knot can be tied in the wire below the interface to insure the bond between the lead wire insulation and the interface from breakage during abnormal stresses. *Note! Care must be taken to allow a sufficient amount of wire below the skin to eliminate stressing the interface during normal movements of the animal. If the wires become taut during movement, the incision will not heal and the interface will eventually be torn loose.*
5. The silicone rubber ridge of the interface is slid down the wires and into place at the implant site;
6. The incision is closed with silk sutures;
7. When it is likely that the animal would claw or chew at the implant site, application of a protective bandage is appropriate;
8. After one week the sutures are removed and under normal conditions complete recovery occurs within the following week.

The Gertler type interface has been successfully used in a number of laboratory animals (adult cats and rats). There have also been a small number of failures when the interface was used. In these cases, it was always found that in certain movements made by the animal, the wire lead became taut. The taut wire (external or internal to the skin) applied a stress to the incision site resulting in connective

tissue destruction and inadequate healing. In the unsuccessful instances, failure occurred within three days of implant.

THE CALDWELL ELECTRODE

The physical properties of the recording or stimulating electrode, to a large extent, dictate the success or failure of an experiment. If the electrode breaks, the experiment is most likely lost. If the properties are such that the electrodes induce excess tissue trauma, intense fibrous encapsulation will result. As the fibrous capsule thickens, the physical separation between the electrode and active tissues increases. The effect of this separation increase is to diminish the amplitude of the recorded electrical signal or to raise the effective threshold to excite the surrounding normal tissue.

The aforementioned difficulties may be minimized by the following considerations:

1. using biocompatible conductors, such as platinum and stainless steel;
2. using a flexible electrode configuration to reduce mechanical trauma;
3. choosing a material and electrode configuration to avoid material fatigue, resulting in electrode breakage.

There are perhaps a number of combinations of materials and electrode configurations which could perform with these points. Based on the experience gathered in this laboratory, the Caldwell electrode [6] has been found to be satisfactory for use in both recording and stimulation applications.

The electrode is formed by winding a steel alloy wire (28μ diameter) around a mandril 110μ in diameter. The end result is an electrode approximately 180μ in diameter which closely resembles a tightly wound helical spring upon microscopic examination. Using an insulated wire and regulating the amount of wire deinsulated, the exposed electrode surface can be controlled. Using metals such as Karma®, 304 stainless steel or 316 stainless steel in this configuration, an extremely flexible and sufficiently strong electrode is obtained. This combination with a silicone rubber filler (which adds strength) has been successfully implanted in animals and man for periods in excess of one year without complications.[6]

The Caldwell electrode is implanted with the aid of a hypodermic needle, usually a 22 gauge. The electrode is placed in the needle with the deinsulated end at the needle tip, leaving a small hook at the deinsulated end. The hook is formed by bending the wire backward over the coiled section, which limits the inward travel and provides a barb to catch in the tissue when the needle is withdrawn. In Figure 3 is shown a typical electrode in the hypodermic needle prior to implant.

The passive tissue reaction (no external potential applied) to this electrode has been extensively studied by Peckham,[7] Caldwell [6] and Gertler et al.[8] The results of these investigators indicate that the average fibrous capsule thickness when implanted in muscle is 90μ with a standard deviation of 40μ.[8]

The tissue reaction to an active electrode during stimulation of skeletal muscle was studied by Gertler et al.[8] In this study, a pulsed DC current that ranged from 1 ma to 15 ma was applied for approximately a twenty-four hour period. The pulse duration was 100μsec at a frequency of 50 Hz. Microscopic examination of the muscle sections revealed no statistical difference in tissue damage between the passive implants and those subjected to the highest current levels in that study. The experience of these authors substantiates the Gertler et al.[8] findings in experiments of up to 31 days of continuous stimulation. (The range of stimulus amplitudes for most physiologic preparations is between 0.5 ma and 10 ma.) As this study was limited to the cathode, it is worthwhile to mention that these results may not hold for an anode with similar physical properties.

CHRONIC IMPLANT TECHNIQUE

The procedure for implanting the chronic stimulating (or recording) system into an animal consists of three steps: injection of the electrode into the muscle, connection of the electrode to the lead wires, and passing the lead wire through the skin interface.

Experiments performed to date have concentrated on the chronic electrical stimulation of



FIGURE 3.—Caldwell type intramuscular electrode in hypodermic needle. The deinsulated portion of the tip is one millimeter.

the tibialis anterior muscle of cats, and this implantation method will be described. A five centimeter incision is made over the belly of the tibialis anterior muscle and the subcutaneous fascia separated by blunt dissection. The deep fascia of the muscle is then opened, exposing the muscle fibers. The hypodermic needle containing the Caldwell electrode is inserted into the desired portion of the muscle. The needle is slowly withdrawn while applying a slight pressure near the tip of the needle, leaving the electrode in the muscle. Connection of the small electrode to a more sturdy lead wire then follows. This is the lead wire which will eventually exit the animal at the interface. The lead wire is a helical coil wound from 130μ type 316 stainless steel wire (coiled diameter 0.72 mm) and insulated with a silicone rubber tubing. (The insulated lead wire was obtained from Medtronic Inc., Minneapolis, Minnesota.) The insulation is pushed back from the tip of the wire to expose at least 15 mm of coiled wire. The coiled wire is then folded back on itself to form a U shape without permanently deforming the coil. At the outside portion of the bend, the coils are open and the free end of the Caldwell electrode can be passed down the center of the coiled lead to exit at the bend, shown in Figure 4. (The tool used in this figure to maintain the bend is formed by drilling two holes in the jaws of the needle holder.) Once the free end of the electrode exits the coiled lead wire at the bend, the lead wire can be allowed to straighten. Pulling the free end of the electrode will draw it into the lead wire. Pulling also strips the insulation from the electrode wire, allowing electrical contact between the lead wire and the electrode. Contact should be tested by applying an external stimulus and observing the twitch response. A drop of Silas-

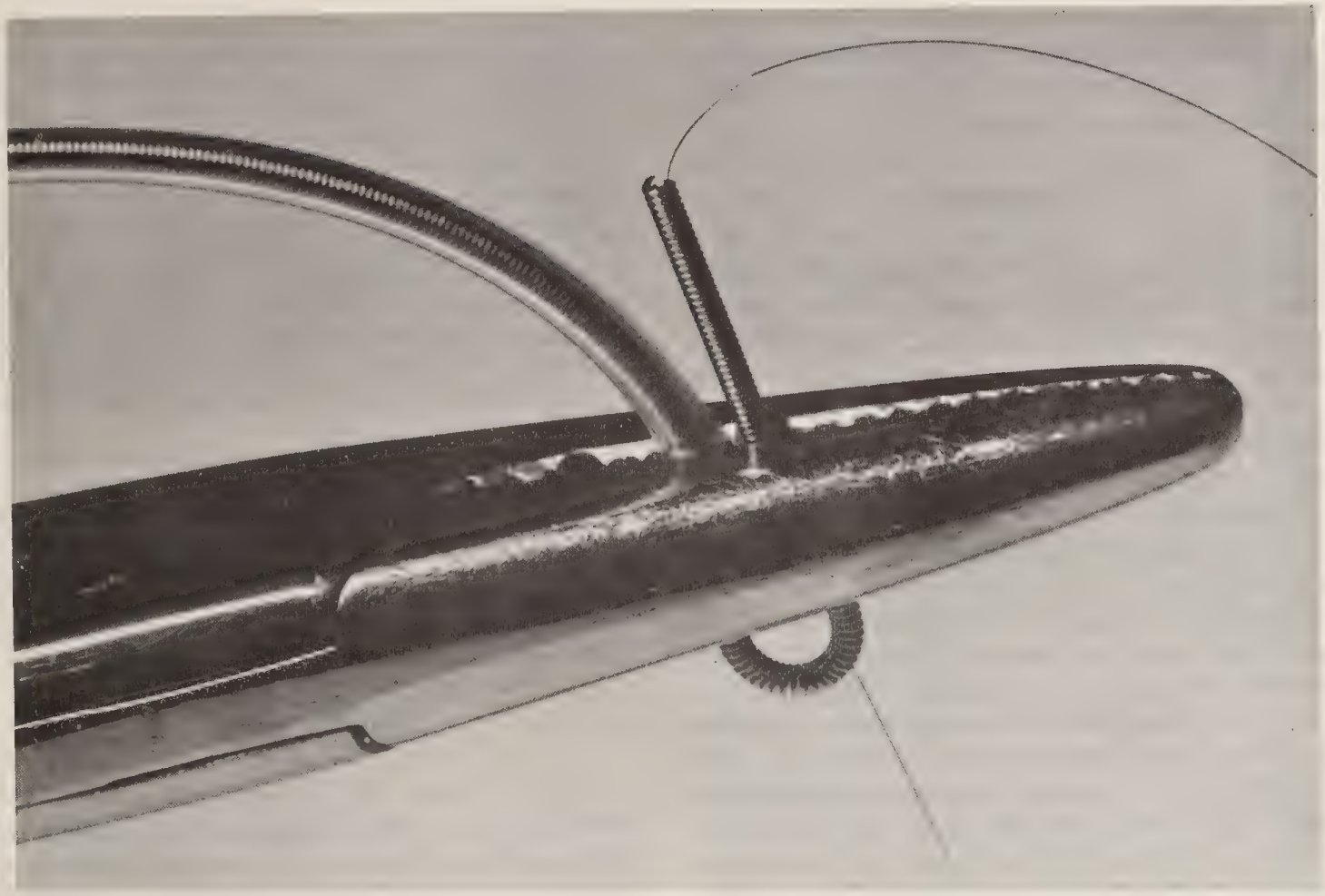


FIGURE 4.—Lead wire coils being held open for connection with intramuscular electrode.

tic Medical Grade A Adhesive is applied to the end of the lead wire and electrode junction. The insulation of the lead wire is then slid down over the connection. The adhesive acts to bond the insulation to the lead and electrode while providing some strain relief for the electrode at the junction. Tying the insulation with 1–0 silk will secure the insulated tubing while the adhesive is setting. The heavier silk is necessary to avoid cutting through the insulation. Two sutures are then placed in the superficial portion of the muscle to secure the electrode-lead junction to the tissue and to prevent accidental displacement. The incision is then closed by first suturing together the deep fascia over the electrode and lead with 4–0 to 6–0 gut suture. Closing the fascia eliminates most relative movement of the superficial tissue over the electrode minimizing the possibilities of electrode dislodgement or breakage. The skin incision is then routinely closed.

Threading the lead wire beneath the skin to the interface area is accomplished by pushing a small blunt rod or probe (available from Codman—Surtleff, Inc.) from the muscle incision to an incision on the back of the animal through the subcutaneous space. Attaching the lead wire to the trailing end of the blunt probe allows the wire to be drawn easily between incision sites. The same procedure is repeated to draw the wire from the incision on the back to the interface incision. This final incision is approximately 2 cm long and is made 1 cm caudal to the inion of the skull. Securing the lead wire to the masculature of the back with silk sutures at the back incision site is advisable as it helps to prevent stress on the lead wire which could dislodge the electrode from the muscle. The anode electrode is usually much larger than the stimulating electrode and can be located at any point on the back of the animal. Securing the anode electrode by silk suture is recommended.

The incisions over the back and electrode site are closed with silk in a routine manner. The interface construction can then proceed as described in the first section.

SUMMARY

Transfer or conduction of electrical signals from one side of the skin to the other may be accomplished by percutaneous wiring. The key points to consider in a successful percutaneous system are: maintenance of an "infection free" interface and minimizing the adverse effects at the recording or stimulating electrode site. Maintenance of an "infection free" interface may be affected by minimizing wire slippage (in and out of the subcutaneous space) and minimizing the size of the fissure between the skin and wire (or anchor). The interface described in this text, designed with these two factors in mind, has been successfully employed in animal experiments which involved cats and rats. To date, it appears that the adverse effects occurring at the stimulating or recording electrode are primarily the result of mechanical trauma inflicted by the electrode. The Caldwell electrode has been found to be satisfactory for use in both recording and stimulating applications. This electrode causes very little tissue damage (in many cases it may be considered negligible) and has been used in experiments involving animals and man for periods in excess of one year without failure. Special care must be taken in connecting the electrode to the main lead wire because of the very fine wire used in the construction of the electrode. A technique has been developed to accomplish this connection which is suitable for surgical implantation.

REFERENCES

1. Morrison, S. J. Tissue reaction to three-ceramics of porous and non-porous structures. M.S. Thesis. Clemson University, Clemson, South Carolina, 1971.
2. Gibbons, D. F. personal communication. Case Western Reserve University, Cleveland, Ohio, 1971.
3. Gibbons, D. F., Peckham, P. H., Martin, R. L. Vitreous carbon percutaneous electrodes for muscle stimulation. Accepted for publication in J. Biomed. Mater Res. Symposium No. 3, pp. 155–164, 1972.
4. Miller, J. Problems related to the maintenance of chronic percutaneous electronic leads and catheters—application of porous ceramic devices. Presented at the Engineering Foundations Conference on Engineering in Medicine—Bioceramics, Henniker, New Hampshire, 1970.
5. Gertler, R. A., Adler, A. G., Mortimer, J. T. An interface for passing lead wires through the skin of laboratory animals. IEEE Trans. on Biomedical Engineering, Vol BME–18, No. 1, pp. 72–73, 1971.
6. Caldwell, C. W. A high strength platinum percutaneous electrode for chronic use. Ph.D. Thesis. Engineering Design Center Report No. EDC 4–70–30, Case Western Reserve University, Cleveland, Ohio, 1970.
7. Peckham, P. H. Design considerations in electrical stimulation of skeletal muscle. M.S. Thesis. Engineering Design Center Report No. 4–68–23, Case Western Reserve University, Cleveland, Ohio, 1968.
8. Gertler, R. A., Mortimer, J. T., Martin, R. L. Effects of intramuscular stimulation on skeletal muscle. (Preparation). Case Western Reserve University, Cleveland, Ohio. 1972.

DISCUSSION

N. P. Ching, St. Vincent's Hospital, New York: Can you tell us what is the duration of your implants, particularly when you took samples for your pathology study of tissue reaction?

Chairman Franklin: Repeat the question please.

Dr. Mortimer: He wanted to know how long we had had the implant in, or when we did the pathology on the stimulation. It was done five days post experiment. At this point one would see the phagocytic activity approximately at its peak, and that's what we were seeing here primarily. The other ones we've seen were cases of continuous stimulation for thirty days. The passive implants have run something like up to three months and that was included in the data.

E. P. McCutcheon, University of Kentucky, Lexington, Ky.: We found this procedure a bit tedious for six or eight leads that we're bringing through, which already have connectors on them. You'd have to build it on-site and that's awkward. One thing we've been trying and had some initial success with is a nylon velour bag that we simply bury under the skin, close it over completely, and then go back in ten days to two weeks. You can window it, overlap the skin, and sew it down. Then you can go back

and unzip or open that bag up and bring out your lead wires, run the animal, then place the leads in and sew it up again. So far this is looking very promising. I'd be interested in other people's experiences with this approach.

DR. MORTIMER: I think that's fine. I think there's a lot of alternate techniques that are certainly going to be appropriate, and the more you have, the more options an investigator will have. For us, if we want to run them continuously, we need to take the data off daily and I think this doesn't lend itself too well to doing that repeated surgery. It is a tedious operation, and using the spring connector has been the way out for us on this. The reason we're forced to use it is the method of injecting this electrode. If we put a connector on it that we can handle, we can't get it in through our hypodermic needle when we try to pull it back through.

THE USE OF ANIMALS IN MEDICAL RESEARCH AND EXPERIMENTATION*

H. Sandler, T. B. Fryer* and H. L. Stone**

All parameters necessary for the description of cardiovascular performance in hydraulic or mechanical terms can be presently obtained by telemetry from units implanted completely inside the body. These include the electrocardiogram (ECG), pressure, dimensions and flow. Single channel ECG units have been implanted in animals ranging in size from a mouse to a Rhesus monkey to a dog. Such units have functioned with continuous transmission of data for periods in excess of one year. Multichannel units have been chronically implanted in dogs and chimpanzees and have functioned for periods in excess of one year. Battery power for multichannel units has been conserved by the use of implantable switches. Multichannel operation is provided by a time sharing multiplex scheme for switching between various sensor inputs. Data are transmitted on a single carrier frequency so that only one receiver is required for recording 5–8 channels of data. Sampling rate is 0.7 msec per channel. Present range of transmission for single channel units (continuous operation) is 3 to 5 feet; multichannel units transmit 50 to 100 feet. A system has recently been developed to recharge the implanted battery using RF radiated to a subcutaneously implanted coil. Recently, multichannel backpack units have been developed for measuring flow using Doppler and electromagnetic flow transducers. Backpack units have also been designed to contain a transponder which receives the weaker transmitted signal from an implanted unit and retransmit the received signals for far greater distances. Distance and duration of transmission with such latter units depend solely on the available power supply in the backpack.

INTRODUCTION

As a consequence of continued advances in bioinstrumentation and microelectronics, single and multichannel systems are now available for transmitting selected physiologic signals from surgically implanted units. Interest in the use of such systems has been strongly stimulated by recent investigations which require information from experimental subjects in a relatively normal physiological and psychological state and under conditions which interfere with normal patterns of activity as little as possible. Biologic use of these systems can now be traced back approximately 15 years and have been made possible by the development of the transistor. This latter device has allowed for construction of telemetry units which are small in size and low in power consumption, leading eventually to units which can be swallowed or implanted inside the body cavity without significant alteration of physiologic function.

The use of completely implantable systems rather than backpack devices for measuring the same functions involves added complications to any experiment. Chief among these are the needs for miniaturization, protection of all components against corrosion by body fluid and high reliability of operation for long periods of time, since they become inaccessible once implanted inside the body. The need for a totally implanted power source, usually batteries, is also a serious design problem since they ordinarily have a limited life expectancy. On the other hand, the use of backpack units which utilize equipment placed on the surface of the body have other problems. These include the requirement that transducer leads penetrate the skin and that the transmitting system be worn in some protected fashion on the body surface if chronic or long-term measurements are to be made. The site at which the leads or wires emerge from the skin frequently become infected or act as a means for body surface bacteria to enter inside the body cavity and are often a source for irritation to the animal and must also be protected; they may often be damaged deliberately or unintentionally by the animal or man. Many protection devices have been designed for the backpack transmitters such as

* NASA-Ames Research Center Moffett Field, California.
** Marine Biomedical Institute Galveston, Texas.

helmets, saddles or submersible canisters. The use of these latter systems requires much more careful surveillance and training of such instrumented animals than when using implanted units. These problems markedly increase in complexity as the number of monitored parameters is increased. Experience in this laboratory has also shown that the use of backpack units becomes very difficult or impossible when uncooperative animals are utilized. As advances are being made in technology and as monitoring needs are increasing in complexity, it is becoming evident that implantable systems have an important and indispensable role in physiologic investigation.

Various investigators have reported implantable systems for measuring temperature,[1] vascular and intraocular pressures,[2,3] brain activity,[4] ECG[5] and acceleration.[6] The number and nature of the systems utilized in a particular experiment are now largely determined by the demands of the experiment, the size of the animal and the nature of the transducer. It is the purpose of this article to discuss the present state of development and use of implant telemetry systems for study of cardiovascular function.

DESIGN REQUIREMENTS

The implant package must be sufficiently small so as not to disturb the normal physiological functioning of the animal. The tolerable size obviously depends upon the size of the animal and the location of the implant package in the body. Multichannel systems, weighing 30 grams and with a volume of 25 cm^3 have been used frequently in 15– to 20 Kg dogs. Although technological advances in microelectronics and integrated circuit techniques make it possible to develop very sophisticated electronic systems in extremely small packages, such methods do not solve the power supply problem or the need for micropower operation if long duration of operation is required. Since the implant system must contain not only the electronics, but also the power supply, the use of the smallest possible battery consistent with the required operating life is essential. The magnitude of the power drain then becomes an important criterion, since it effects the overall size of the implanted unit through the battery requirements.

A power requirement of a few microwatts to a few milliwatts has been achieved in the single channel unit designs. Sensors vary considerably in their power requirements depending upon the kind of measurements being made. High impedance thermistors require only a fraction of a microwatt for temperature measurements. About ½ milliwatt is required to operate the solid state, strain-gage type cells presently used for pressure measurement, while the transducers for flow measurement require considerably more power.

The other major part of an implanted telemetry system is the radio-frequency (RF) transmitter. Power requirements depend upon the operating range, antenna efficiency, information rate, and encoding scheme used. To reliably sustain oscillation in the transmitter state, a 2N918 type transistor has been used in the circuit designs in this laboratory and requires a minimum current of 0.5 to 0.8 ma or more, using a 1.35-volt mercury cell. This power level will typically provide an operating range of 50 to 150 feet which is more than adequate for most experiments. In order to reduce the average transmitter power demand to the microwatt power level, a pulse modulated RF is used instead of a continuous wave (CW) carrier. This results in a reduction of range to 5 to 10 feet because of susceptibility to spurious noise, but provides continuous transmission of data and is adequate for use with small caged animals, such as rats.

The use of glass, metal, or ceramic to provide hermetically sealed cases for all the electronics is the only reliable method for protection of a totally implanted transmitter. Metal with glass-to-metal seals is the most versatile construction technique, but special precautions must be observed to obtain effective RF radiation. Ceramic and glass will not interfere with the RF, but the high sealing temperatures can cause difficulties with the electronics and batteries.

METHODS AND RESULTS

All parameters required for description of cardiovascular performance in hydraulic or me-

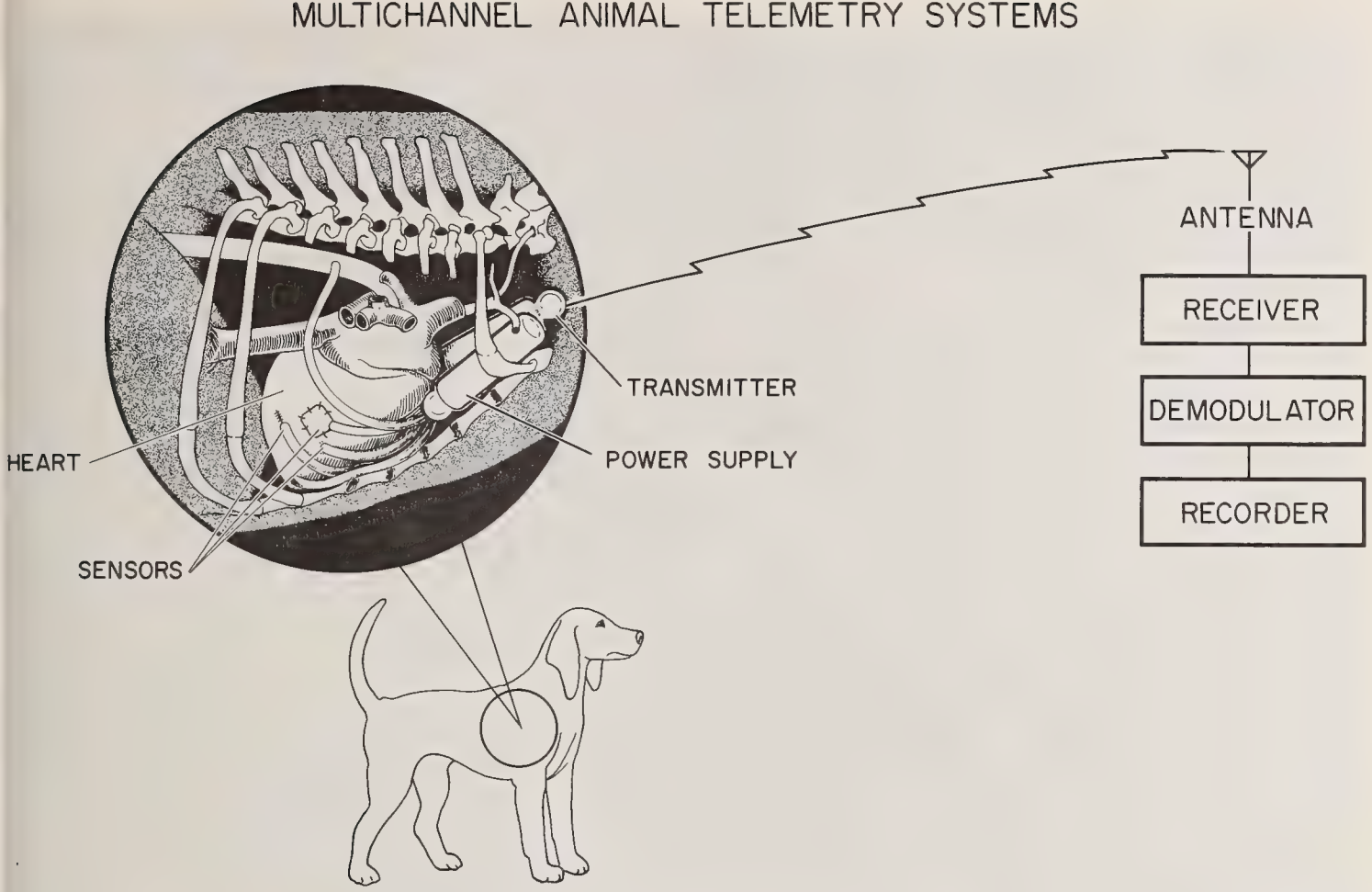


FIGURE 1.—Multichannel animal telemetry systems.

chanical terms can presently be obtained by telemetry. These include the electrocardiogram (ECG), pressure, dimensions and flow. In a typical system the appropriate analog signal resulting from the transducer is placed in a form or code for transmission, transmitted by radio wave and then received and decoded in its original form at a receiving station for subsequent storage and analysis. This process is schematically illustrated in Figure 1. The choice of operating frequency, transmitting and receiving antennas, modulation scheme and receiver are dictated by the demands of the experiment. Data is transmitted using a subcarrier which is an important feature since it allows for transmission without significant loss of accuracy. The most commonly used schemes for these latter purposes are frequency modulation (FM), pulse interval modulation and pulse code modulation.

A schematic view of a single channel ECG transmitter is shown in Figure 2. These units measure 2.5 cm x 1.2 cm x 0.5 cm, including battery, and are constructed from discrete parts. They have been used in animals ranging in size from a mouse to a dog and have operated continuously for over one year under these circumstances, utilizing a single 1.5V mercury cell battery. Recently a small atomic source of power (promethium) has been used to replace the mercury cell battery.[7] The anticipated life expectany of these units is 2 to 3 years of continuous operation.

Single channel pressure,[3] dimensional[8] and flow[9] units have been developed and tested. The pressure transmitter is comparable in size to the ECG unit and uses a small strain gage-type cell as shown in Figure 3. The useful life of these units has been 6 weeks of continuous operation which has been extended to 6 months or more by intermittent recordings when an implantable switch has been incorporated as part of the battery pack.

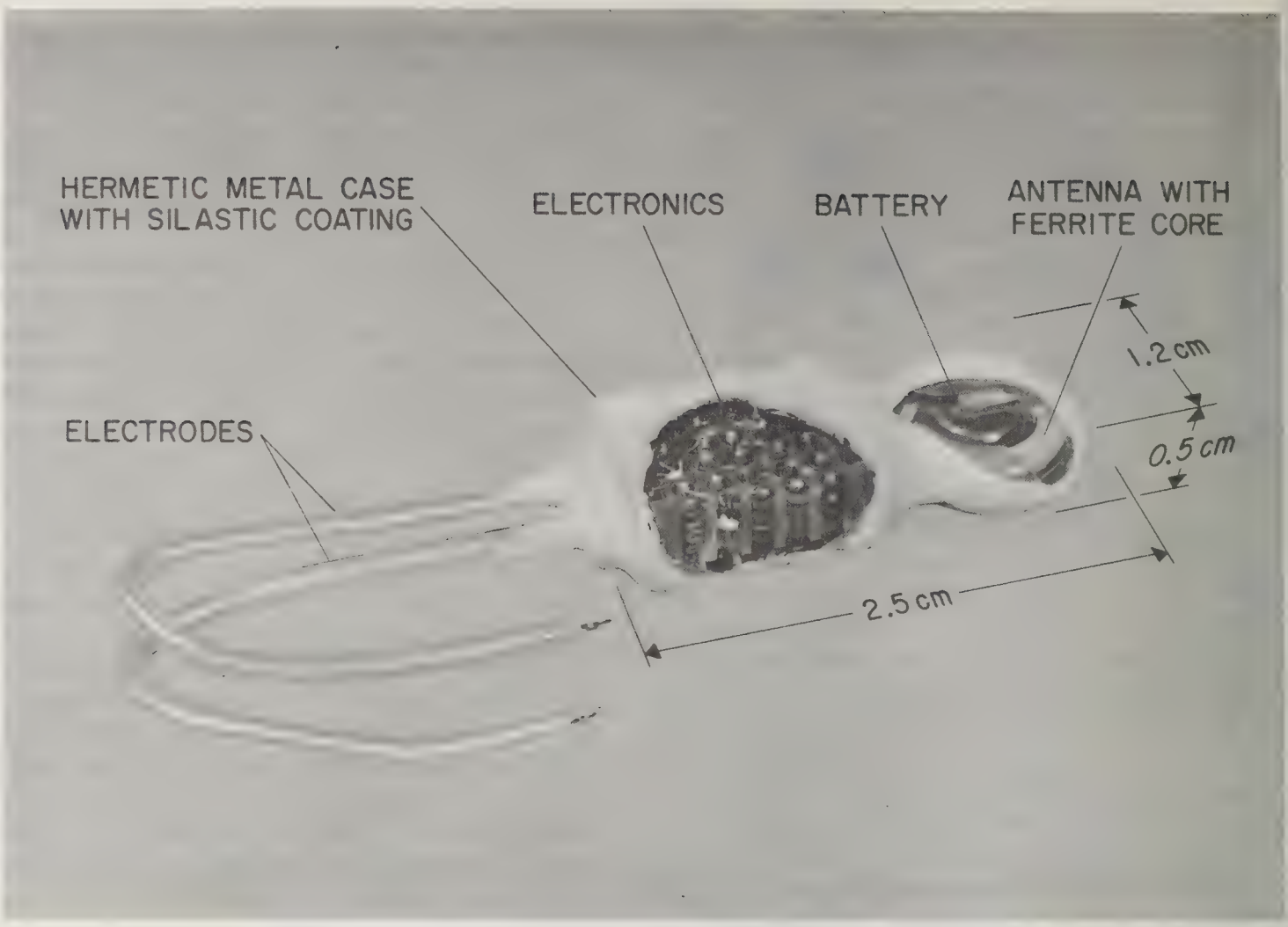


FIGURE 2.—Single channel implantable ECG unit.

Studies of cardiovascular phenomena in awake animals have demonstrated the need for monitoring of more than one or two variables. Although multiple single channel units may be used for such purposes, a system which uses only a single RF link and therefore only one receiver greatly reduces the complexity of such studies. A system capable of transmitting 3 to 10 channels of data on a single RF link has recently been constructed and tested in our laboratories and proved to be reliable and reasonable to use.[10,11] The pulse generation and RF transmitter circuitry are similar to that for single channel units. By careful choice of circuit design, power consumption can still be maintained at very low levels. Multichannel operation is provided by a time sharing multiplex scheme for switching between various sensors. Sampling rate is 0.7 msec per channel. A typical 8-channel system, illustrated in Figure 4, consists of three pressure sensors, two ECG leads and a three-axis accelerometer. For four pressure transducers and one set of ECG electrodes the average current drain is 2 ma from two mercury cells (1.35 V each). By the use of an implantable switch 500 hours of continuous operation has been extended to over one year.

Multichannel capability has also been developed to detect flow and distribution of flow using Doppler and/or electromagnetic flow (EMF) transducers.[12,13] The development of a multichannel battery operated EMF system has been of considerable interest because of its accuracy and wide acceptance for cardiovascular research. Provision has been made in the design to handle as many as four simultaneous flow measurements. Batteries and electronics for a four-channel system weigh approximately

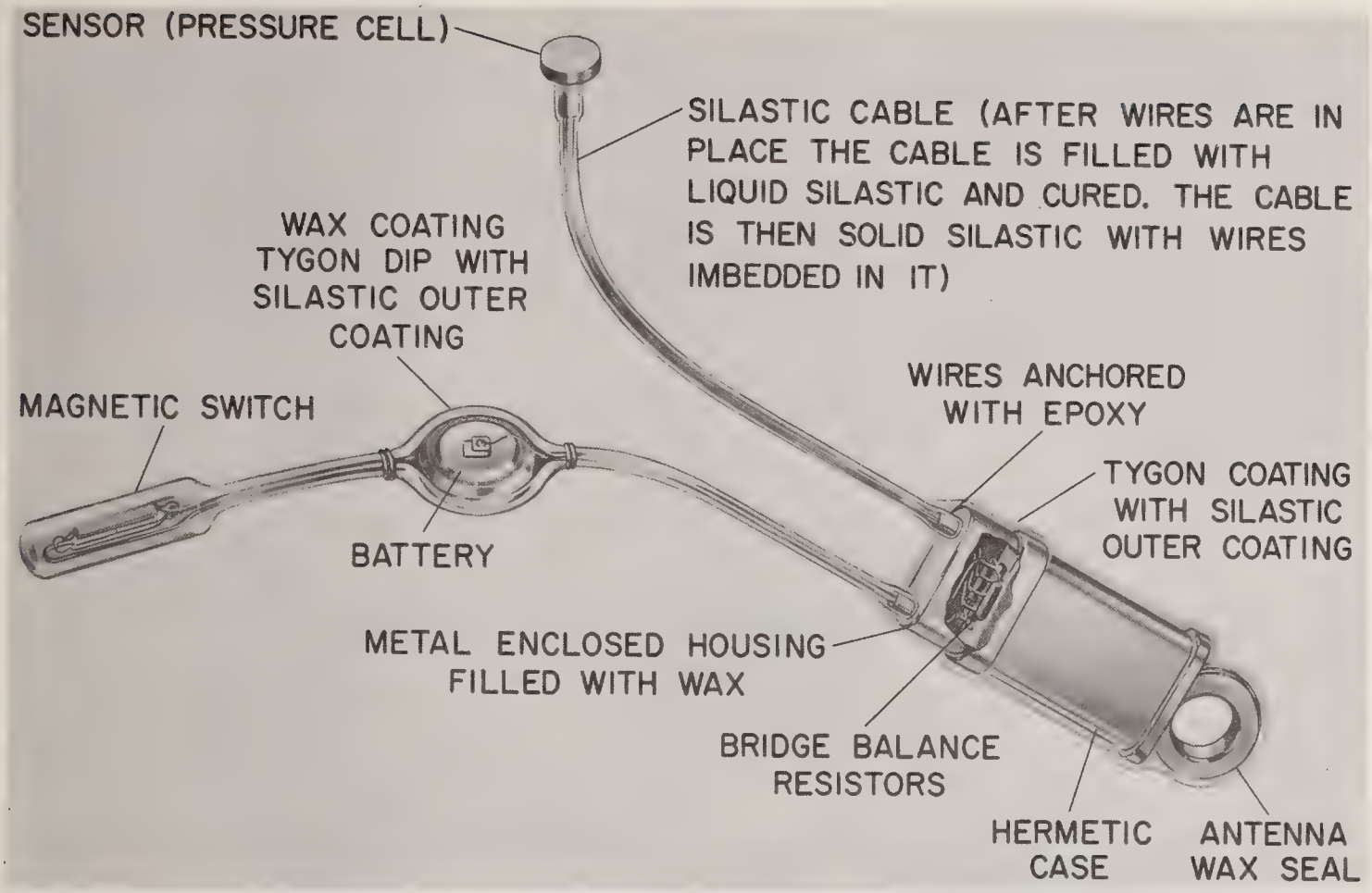


FIGURE 3.—Schematic of single channel pressure transmitter.

500 grams and have a volume of 250 cm^3. A typical recording of flow including pressure and ECG is shown in Figure 5. This system has been used to measure cardiac output and flow distribution in unrestrained animals in the laboratory and in the field. Ten hours of continuous operation are provided by two rechargeable nickel cadmium batteries. An implantable recharging system has been developed for such systems utilizing transfer of electromagnetic energy through a closed chest wall.[14] The system consists of an implanted coil of teflon coated wire 4″ in diameter. Energy is transferred through the skin from an external coil of the same diameter 3–5 cm away. The recharging system has been used successfully for up to six months with the multichannel pressure telemetry units described above. It is presently being evaluated for powering a single channel completely implanted EMF unit.

DISCUSSION

The selection of proper instrumentation to measure various physiological parameters is an important aspect of any experimental research program. Measurements can be made either by direct connection to a given recording system or by means of radiotelemetry. For most acute experiments, direct connection is least costly, most easily implemented and usually most satisfactory. For long term experiments requiring data from awake unrestrained animals the use of a telemetry link is essential. Recent developments in microelectronics have allowed for the development of useful chronically implantable telemetry units. The requirements of small size and reliable operation in the hostile saline environment of the body adds complications to any experiment. These problems are rapidly offset by the advantages of their use when multiple parameters are to be measured over long peri-

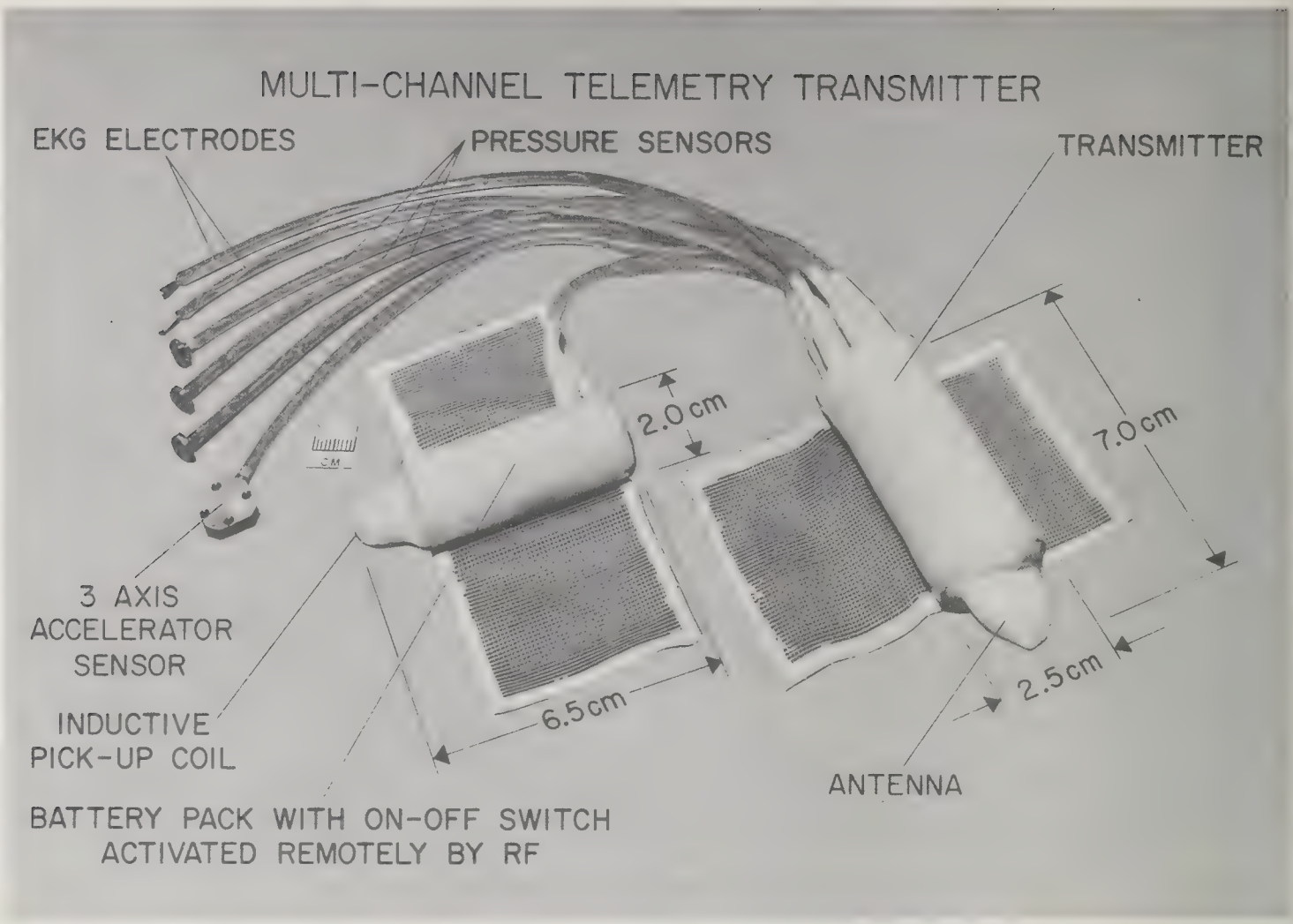


FIGURE 4.—Multichannel telemetry transmitter.

ods or when a highly uncooperative animal is being utilized.

Since the power supply, which is usually a battery, must also be implanted, the selection of circuits that consume only microwatts of power, or at most a few milliwatts, is essential. Each experimental design must be evaluated to determine the best tradeoff between battery size and such factors as telemetry range and operating life of the unit. The selection of transducers is also a major factor in determining such power requirements. Using single channel temperature or EKG units as little as 12μW of power are consumed while continuously transmitting data for a distance of 3 to 5 feet. A multichannel system with four pressure transducers consumes 6mW to transmit 50 feet. A single channel electromagnetic flow system consumes 400 mW to transmit data the same distance.

An important feature of the designs used in the described units were their ability to attain a great degree of accuracy utilizing sophisticated modulation techniques. The use of subcarrier oscillators and automatic calibrations are the main factors responsible for these results. The methods used for the implanted units are essentially identical to those used for the larger powered telemetry systems developed for space research. This allows the accuracy available from the selected transducers to be transmitted over a radio telemetry link without degradation.

These devices in their present state of development have a variety of potential applications distinct from their present use in a laboratory environment. Appropriate units could be used in animal tracking studies which require physiologic measurements, as well as information

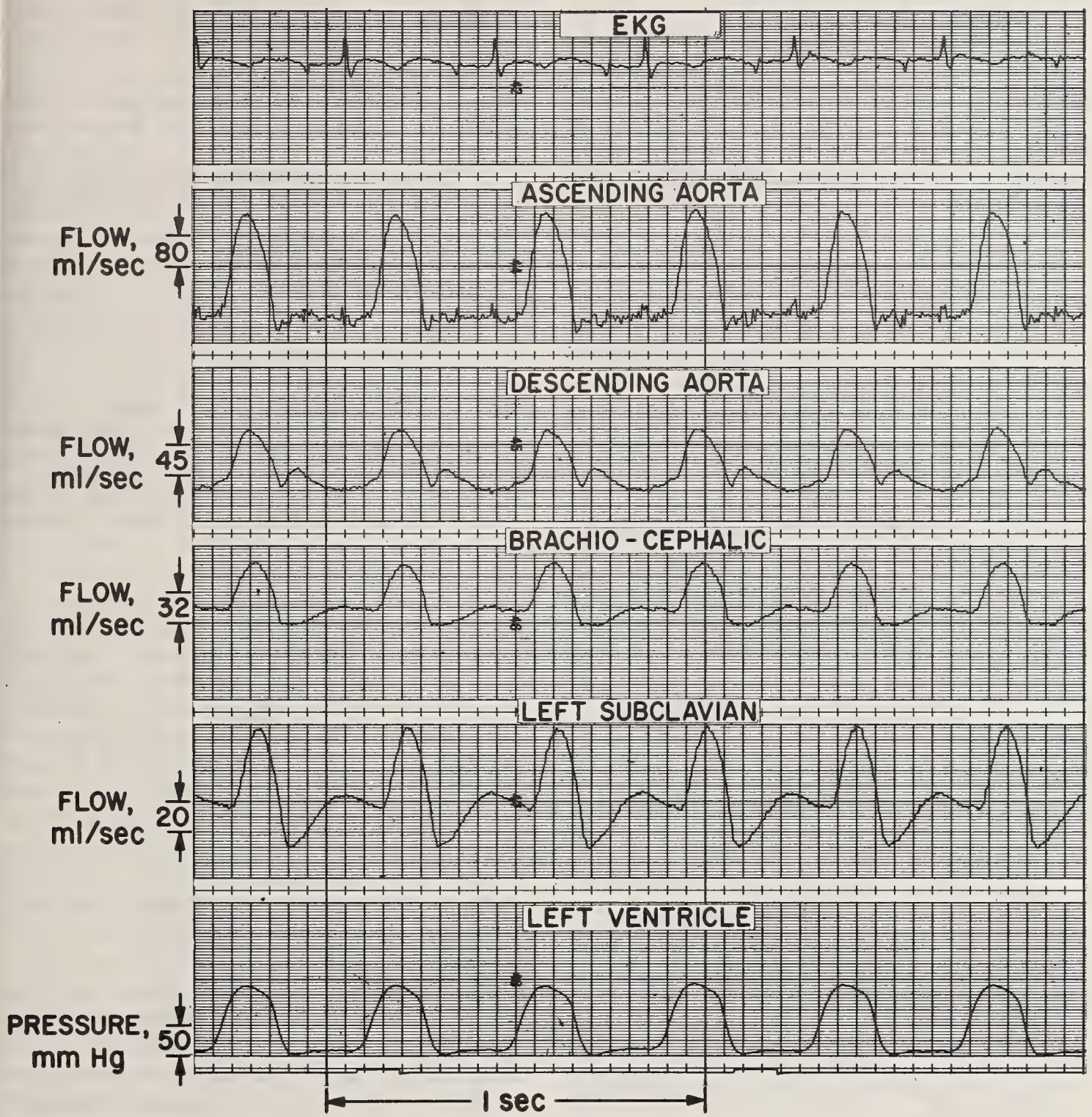


FIGURE 5.—A typical recording of flow from backpack electromagnetic flow telemetry system.

concerning location. Studies are being conducted to determine the feasibility of operating these units as passive transmitters (energy is received from an external source), rather than using an implanted power source. This source may be a backpack worn by the animal. Work is already in progress, as mentioned above, to replace conventional power sources with more reliable and long-term sources of power such as radioactive materials. Tests need to be conducted and evaluated in using small retransmitters or transponders which receive the signals

from smaller implanted units and retransmit the received signals for long distances. Lastly, implantable units have potential applications directly for use in man. Examples of such applications would be electromographic transducers to control artificial arms or legs, blood vessel clamps, controllers for an artificial heart and ECG and intravascular pressure sensors.

SUMMARY

Single and multichannel implantable telemetry systems have been developed and tested in various animals.

Such units have remained operational with continuous or intermittent data transmission for periods in excess of one year.

Systems have recently been developed which allow for measurement of flow and dimensions as well as ECG and pressure.

Although implanted systems may add complexity to many experiments, they are proving to be invaluable for situations requiring long-term monitoring of multiple parameters or when uncooperative animal subjects are involved.

REFERENCES

1. Fryer, T. B., Debor, G., and Winget, C. M. Miniature long life temperature telemetry systems. J. Appl. Physiol. 21:295–298, 1966.
2. Collins, G. C. Miniature passive pressure transensor for implanting in the eye. IEEE Trans. Biomed. Eng. BME 14, pp. 77–83, 1967.
3. Sandler, H., Fryer, T. B., and Datnow, B. Single channel pressure telemetry unit. J. Appl. Physiol. 26:235–238, 1969.
4. Deboo, G. J., and Fryer, T. B. Miniature biopotential telemetry system. Am. J. Med. Elect. 4:138–142, 1965.
5. Fryer, T. B. Implantable biotelemetry system developments at Ames Research Center. NASA Technical Report SP 5094, 1971.
6. Sandler, H., Fryer, T. B., Westbrook, R. M. and Konigsberg, E. Miniature Implantable Accelerometers. In: *Implanted Cardiovascular Instrumentation.* E. P. McCutcheon, Ed. Academic Press, Inc., N. Y. (in press) 1972.
7. Kolnek, S. A., and Hursen, T. F. A reliable nuclear battery for cardiac pacemaking. Proc. Conf. Eng. Med. and Biol. 12:286, 1970.
8. Lee, R. D., and Sandler, H. Miniature implantable sonomicrometer system. J. Appl. Physiol. 28:110–112, 1970.
9. Cook, G. R., and Sandler, H. An implantable ultrasonic telemeter for the measurement of blood flow in animals. Proc. Conf. Eng. Med. and Biol. 12:281, 1970.
10. Fryer, T. B., Sandler, H., and Datnow, B. A multichannel telemetry system. Med. Res. Eng. 8:9–15, 1969.
11. Sandler, H., Stone, H. L., and Fryer, T. B. The use of implantable telemetry systems for study of cardiovascular phenomena. Circ. Res. (in press) 1972.
12. Rositano, S. A., and Sandler, H. Multichannel Doppler blood flow measurement system. Proc. Conf. Eng. Med. and Biol. 11:10.9, 1969.
13. Fryer, T. B., Sandler, H., and Doane, D. H. A multichannel electromagnetic flow meter telemetry system. Proc. Intl. Sym. Biotelemetry. Nijmegen, The Netherlands, May 5–8, 1971 (in press).
14. Fryer, T. B., and Sandler, H. A rechargable Battery System for Implanted Telemetry systems. Proc. Conf. Eng. Med. and Biol. 9:129, 1970.

DISCUSSION

Chairman Franklin: I might comment that we're sufficiently impressed with Tom's success in use of the electromagnetic flowmeter, particularly at the ascending aorta, that we're building one as well. This simple CW Doppler flowmeter is more subject to error and I must say I'm looking forward to finishing that up. We're using Tom's circuitry.

P. Stein, University of Oklahoma, Oklahoma City: How do you establish zero with your flow transducers when they're located at sites other than the ascending aorta?

Dr. Fryer: The results with the square wave have been very good, although this always is a problem using any type of flowmeter. In our laboratory where individual flows were measured and compared, for the ascending aorta and the three main branches arising from the aorta we were able to obtain good results. We have no unique method for determining zero. To get a zero many people have used various flow occluding or blocking techniques. They are difficult to apply in awake animals. Recent studies where we have tested vasoactive drugs or performed ventricular pacing have resulted in episodes of several skipped beats or periods of aseptole on withdrawal of the drug or pacing.

These periods have allowed for zero determination to as accurate a degree as occluders. Often in the resting dog long periods of diastolic filling occur due to normal sinus arrythmia and provides a good check as to whether or not you're approaching a good zero. The basic system has very good zeros with the square waves in single channel checks at the bench and when we've done acute experiments. But like any system, it's not perfect, and it's never as good as you want it.

C. GRANT, Veterans Hospital, Albany: I'd like to ask about the state of development of this from the point of view of consumers. Are there suppliers to whom somebody without special high quality electronic facilities can go and buy a telemetry device for electrocardiogram and pressure recording?

DR. FRYER: We have contracted with a number of people at various times who have been interested in the commercial market. Currently, we have been getting some work done by Konigsburg Instrument Company which makes the pressure cells. Possibly if you contact them, they might be in a position to supply the types of systems you're talking about.

D. PURDY, ARCO Nuclear Co., Pittsburgh: You mentioned that one of your limitations may be power. As you know, the radiosotope power cardiac pacemaker program has developed a nuclear battery for pacemaking, and I wonder if you had considered that type of power for some of your applications?

DR. FRYER: Yes we had. In fact, just two weeks ago, we got in two nuclear batteries that we've just attached to two transmitters. I might mention that they cost $3000 per battery, so I don't think they're very practical at this time. We are testing the system with the hope that they'll be available at a much more modest cost in the future.

I should mention now that you've brought up the question of power supplies for the electromagnetic flowmeter, is considerably greater than for the other systems. I didn't really have time to go into it during the presentation. However, we are using rechargeable batteries, and we're using the type of system that they have also used in the artificial heart program, which allows for recharging the batteries. Our power requirements however, are so much lower than those for a heart so that we can get four to five hours from two pen-light size cells. With periodic recharging this system can be used over and over again. I also should mention that we're currently in the process of assembling a totally implanted flowmeter with pressure and EKG measurements in a multiple channel system.

A. SCRIABINE, Merck Institute, West Point, Penna.: For how long were you able to keep your systems functioning inside of the animals? This is the first question. The second question is about calibration and sensitivity. How did you correct for zero in your pressure cells?

DR. FRYER: Well, let me answer the second question. As far as the calibration of the pressure cells, for zero, we do this as have others by insertion of a catheter. Calibration as far as sensitivity, which normally doesn't change so much, can be done with a chamber that will allow you to change the atmospheric pressure since these are sealed cells. You can get a dynamic calibration that way and we have done that. The only way I know of obtaining zero calibrations for long periods would be from a chronically implanted catheter. We have found very little drift over weeks and months. They stabilize, but you would want to periodically check them at least to verify zero. The other question?

DR. SCRIABINE: How long were you able to keep them functioning?

DR. FRYER: This has been a fairly long-term program in that we've made a lot of measurements with pressure but without the flow. Initial units were manufactured by hand, more recent units are utilizing integrated circuit techniques. We have had systems that are nonfunctioning after a couple of months or so, others have functioned in excess of one year. We have three dogs that have lived in excess of four years with the hardware in them. These animals have the hardware implanted in the chest cavity. They have pressure cells in the right and left ventricles and the aorta, and these dogs are perfectly normal and healthy. I haven't mentioned the fact that these units have their greatest application with those animals that are not very cooperative. We do work

a lot with dogs, but more recently have worked with primates and have placed a number of transmitters in chimpanzees. We have one chimp transmitting excellent data now for over a year.

T. REICH, New York University, New York: To say that Tom's apparatus is elegant would be saying very little indeed. We have had a chance to speak with him about a flow transducer which we're now developing and which may help overcome some of the problems that were presented here this afternoon. The unit that I'm talking about uses a permanent magnet so that many of the power problems will be eliminated. The major problem now that faces us rather is to document that we can keep these units from drifting and we hope that perhaps in the next three or four months that will be accomplished also. I think that with that information there will be a long step forward.

DR. FRYER: Thank you. Yes I think that people of course have used in the past permanent magnets. I have had no experience so I really can't comment. I know that the polarization potentials that one gets at the electrodes of course causes serious problems but I think there is always room for improvements with new techniques. It may be possible to compensate for these potentials and possibly get good flow measurements that way.

D. B. JACKSON, Abbott Laboratories, North Chicago: What is your capsulating material that works so well for your hardware?

DR. FRYER: People that have been around a few years, and involved with electronics, know that the vacuum tube had its essential components in a sealed glass container. Later, transistors often came with glass, although the recent plastic coated ones have, in effect, a glass coating on the components themselves. We've put all of the electronic parts inside a metal container and bring the leads through with the usual type of glass to metal seal using Kovar leads that come with crystal cans. The transducer leads are sealed in Silastic. Recently we have been using stainless steel lead wires for the transducers to avoid corrosion problems and to eliminate lead breakage.

THE ADAPTIVE CONTROL OF THERAPEUTIC PROCEDURES

N. P. Thompson,* B. Widrow** and C. Schade**

If one wishes to optimally control any system which exhibits a nonexplicit time variable delay and a nonexplicit time variable response (gain), he must consider adaptive control.

The control approach being used models the animal's response by means of a tapped adaptive transversal filter. Each tap is separated from the next by a delay. The output at each tap is weighted and summed with all of the other tap outputs. Thus when there is a change in the drug rate being administered to the animal, an equivalent scaled voltage analog change is introduced into the filter. As the response appears in the animal's blood pressure, it is compared with the summed output of the filter's taps. If there is disagreement in the two outputs, the filter's weights are adjusted (adaption) to bring the two outputs into better agreement. After several passes of the filter, its response will be a faithful model of the animal's response. When an error develops between the blood pressure desired and the actual blood pressure, the "inverse" of the model's transfer function is taken to determine what should occur with regards to the vasopressor's administration to achieve the desired change in blood pressure. This change is then implemented. The animal's response to this change is automatically employed to up date the model if necessary.

This procedure can be applied to situations having more than one input and response. In such cases, it may optimize one response while holding the others within acceptable bounds by causing appropriate changes in the therapeutic agents being administered.

INTRODUCTION

There are several ways one might approach the automatic control of therapeutic agents. First, the operator may use the controller as a simple switch, closing the loop and carrying out all of the analyses himself. Thus he may decide that a given drug should be administered for a set period at a specified rate. The controller merely carries out these orders. Such a process hardly deserves being called a control system. It has the advantage that the controller is not free to do much; so the faint of heart and distrusting might find such an approach appealing. Of course, such a controller is also of little help.

The next higher level of controller that one might choose closes the loop using some simple unchanging algorithm. This algorithm might consist of some heuristic plan. For example, if A less than B, give 25 cc of C, or it might be a deterministic function such as seen in ordinary control systems. Here the result (output) is compared with the desired result and an error value determined. This error is added to or subtracted from the input to the system under control thus bringing the output back to the desired level. Such control systems have the advantage that one need only ask for a specified response and the controller will hold that response, if possible. The problem with such simple controllers is that in order to assure stability they must be very slow (as slow as the worst case). Therefore, they cannot hope to be optimum except in the worst case. This objection also holds for the simple approach mentioned first where the operator closes the loop himself if he doesn't wait long enough before making a control decision, instability will result. In addition, such approaches as these must assume that the biological response will be maximal to assure stability. So also on this second basis such simple control systems cannot be optimum except in the best case. Thus such simple control systems can never be optimum because in the worst case the gain will not be optimum and in the best case the delay will not be optimum.

To summarize, one cannot have optimum and stable control in the face of a nonexplicit time variable delay and/or gain unless he is prepared to alter his control strategy to fit changes that occur in the gain and delay of the system

* Palo Alto Medical Research Foundation Palo Alto, California.
** Stanford University, Stanford, California.

being controlled. A control system that is capable of implementing such changes is said to be adaptive.

THE ADAPTIVE CONTROL OF THERAPEUTIC PROCEDURES

We chose to initially study the adaptive control of intravenously administered vasopressors. The decision to start with the control of blood pressure was based more upon engineering than upon medical expedience. Blood pressure can be measured accurately and reliably, and it can be altered by a number of rather specific drugs. For these reasons, by choosing to control blood pressure, one could get on with the central control problem and not spend an inordinate time struggling with transduction and pharmacological questions.

Before proceeding, let us further define adaptive control. With simple control one attempts to maintain a given output by comparing some function of the output with a desired value any difference being the error, Figure 1. This error is used at the system's input to correct the output. It can be shown that such a system will be stable under a prescribed set of circumstances. It is potentially unstable if the plant delay or the open loop gain [(plant gain) × (feedback processing gain)] exceed prescribed limits. If these limits are known and fixed, they can be accounted for in the controller's design, but if not, some other allowances must be made.

By making the feedback processor variable, Figure 2, and allowing it to observe the dynamics of the plant, one can hold the open loop characteristics within prescribed bounds. Such a system is said to be adaptive. Adaptive systems come in many forms, but they all have two things in common. One, somewhere within the controller there is a means of identifying and

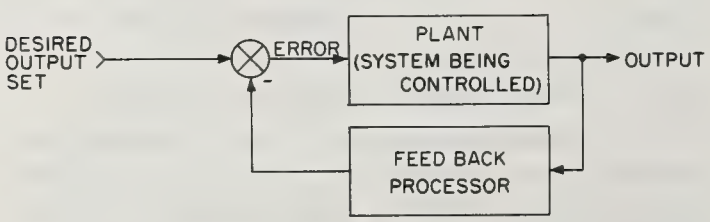


FIGURE 1.—A simpler linear control system.

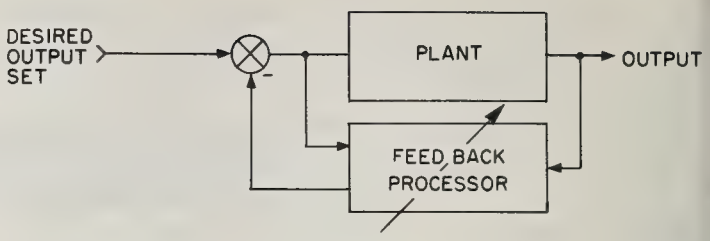


FIGURE 2.—The system in Figure 1 with provision for adapting the feed back loop.

measuring the pertinent parameters in the plant, and two, there is a means for affecting the system's open loop response.

At first a heuristic approach was used in an attempt to realize the system in Figure 2. This worked well for gain adaption, but due to natural fluctuations in blood pressure the end point of a response was hard to locate thus making delay adaption difficult. Correlation techniques could be used, but a better approach was at hand. It not only would allow for gain and delay adaption but also for modeling of the system's total dynamics.

In this approach an "Adaptive Model" identifies the parameters of interest, and a "Foreward Time Calculation" affects the system's response, Figure 3.

The Modeler observes the I.V. Controller/plant response and develops a mathematical model of these two components. The model weights are used by the "Foreward Time Calculator" to determine what X_j should be to obtain r_j.

At first glance it would appear that this is not a control system. No error is apparently determined. As we will see, internally, the model's output is compared with the plant's output. The difference between these, that is the error, is

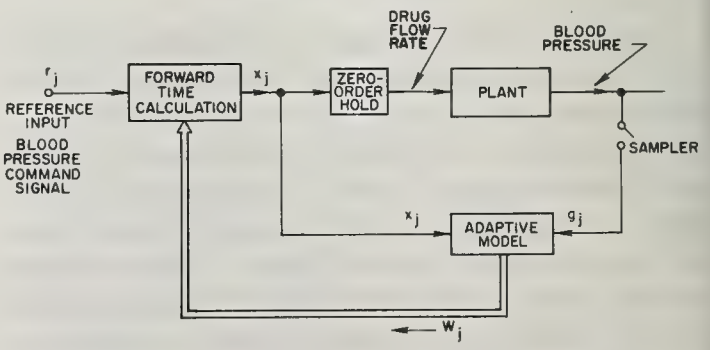


FIGURE 3.—A block diagram of the system now in use.

corrected by correcting the weights and as these weights are in turn used for controlling the plant, we are in fact generating an error function and using it to correct the system's output.

The heart of the modeler is a transversal filter. The main element in such a filter is a tapped delay line. Each tap along the line is connected to a weighting element capable of weighting the tap's output over a range of plus and minus values.

The output of each weight element is fed into a summer and summed with the others. One other element enters the summer, this is a weighted bias. The output of the summer is compared with the output of the system being modeled while the input to the delay line is always a scaled analog of the input to the system being modeled, Figure 4.

After each delay period, each weight is adjusted according to the Widrow-Hoff[1,2] α-LMS algorithm:

$$W_{j+1} = W_j + \frac{\alpha X \epsilon_j}{||X_j||^2} \qquad (1)$$

where

$||X_j||^2 = X^T_j X_j$

$\epsilon_j = g_j - X^T_j W_j$

and

W = the weight coefficient
α = the convergence factor
ϵ = the error, i.e., the Model's output subtracted from the Plant's output at the j^{th} instant
X_j = the total input (to the weights) vector at the j^{th} instant
g_j = the j^{th} sample of the plant's output

It can be shown that when the input and plant are stationary, the weights will converge as long as

$$0 < \alpha < 2.$$

In practice we use $\alpha = 0.4$. α controls the amount of error removed at each update of the weights and thus the rate of the model's convergence. The value must be chosen to assure stability in the weight determination on the one hand and a reasonable settling time on the other.

Figure 5 shows the adaption of a set of weights starting from those of an "average response" and converging to those of the dog under control. To save time in the initial set up period, we begin with an average set of weights for a dog rather than, say, all zeros.

Having generated this model, one can readily predict the response to a given input vector.

$$\text{Output} = X^T_j W_j \qquad (2)$$

The real problem arises in trying to determine X_j, the instantaneous input value (i.e., the vasopressor I.V. rate) at the j^{th} instant. Several approaches are being studied. All seem

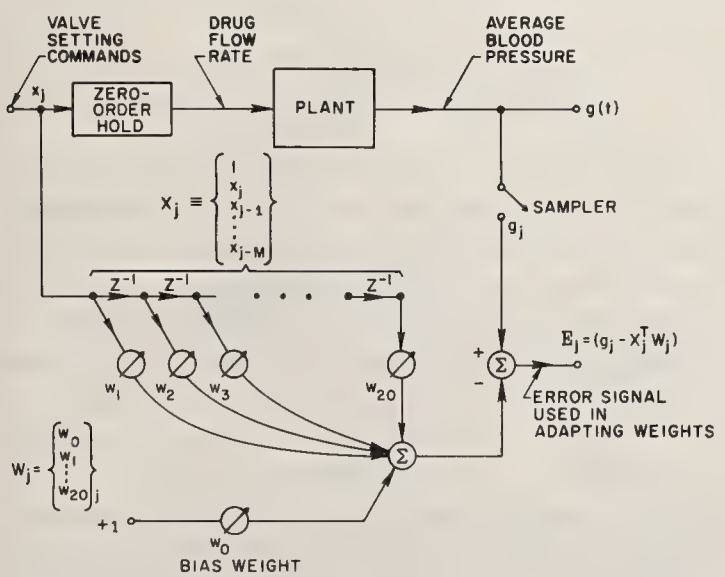


FIGURE 4.—A detail of the transversal filter.

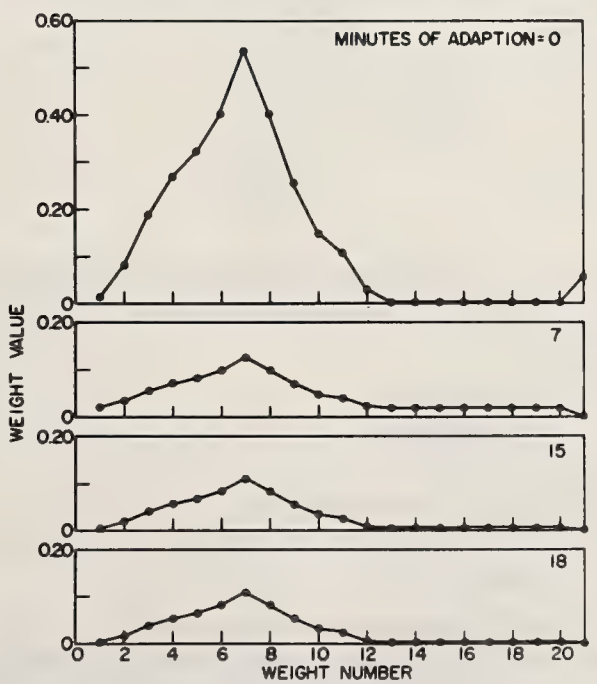


FIGURE 5.—A typical adaption sequence of weights.

to work, but each has its own advantages and disadvantages. In general the more precisely one wishes to maintain control the more it will cost in terms of system complexity. We are studying the various trade offs involved with several approaches now.

One simple approach is to let the model indicate through its weight profile when the response is over, thereby using it as a means of measuring the delay.

One can then measure the response to a given change in input after the delay as indicated by the model. From this response one can determine the system's gain. By storing the gain and delay thus determined and updating them after each control attempt, one can realize adaptive steady state control. This would be a means of solving the problem of delay adaption in the case of our initial heuristic attempts to adaptively control blood pressure. Such a simple approach would not utilize this model's ability to begin adaption before a response is complete, and therefore, it would adapt more slowly than necessary.

To avoid this objection, we arrive at steady state control in a different manner. To state this more succinctly, note that one can determine the required steady state input by the following relationship:

$$X_{ss} = \frac{\frac{1}{LN}\sum_{i=j+1}^{T} r_i}{\sum_{k=1}^{N} W_k} \qquad (3)$$

where L = an integer constant, the delay between taps in the delay line, we currently use 5 seconds
N = number of taps
j = current time
T = LN + j
This solution we call phase I.

One then uses X_i as input values based upon the relationship:

$X_i = X_{ss}$ for all i = j + 1, j + 2 . . . T.

where X_i is the value actually used and X_{ss} is the value computed. This solution may never be optimum, but it will always be stable. It will be better than any nonadaptive approach because of its ability to determine the steady state gain and delay and use these in predicting drug response.

To expand on this last point, note that even though this is called a steady state solution, the weights can be varying from sample time to sample time and with each of these changes a new X_{ss} will be determined. Thus, the only thing steady state is the desired end result. The steady state solution takes the system under control from one steady state setting to another as commanded. How it goes from one level to the next is not determined.

In order to cause the system to go from one steady state value to another as quickly as possible (or in any prescribed way), the plant's dynamics must be used. If we make the transition follow the input command as closely as possible we have, by definition, optimum control. In mathematical terms, optimum control attempts to minimize the function:

$$(y_i - r_i)^2$$

where r_i = instantaneous, desired output
y_i = actual output of the control model
$y_i = X^T_i W_i$

In the next phase, phase II, one sets

$(y_i - r_i)^2 = 0$ for all i = j + 1, j + 2 . . . T

X_i now equals X_i in

$$X_i = \frac{r_i - \sum_{k=2}^{N} W_k X_{i-k+1}}{W_1 *} \qquad (4)$$

so long as X_i is within bounds. These bounds are limits set upon the range of the input function by practice (e.g., no negative IV rates and none above, say, 50 drops/minute). If X_i is within bounds for all i, an optimal solution is possible and we can stop. That is, one can use this value for X_i at each point in X_i where i = j+1, j+2 . . . T and he will have an optimal result. Phase II control is rarely possible.

If X_i does not maintain itself within bounds, it must be limited. Studies yet not implemented

* Where W_1 is the first non zero weight and the delay line has $N-N_0$ elements where N_0 is the number of zero valued weights.

in the controller show that it is possible to compute an optimum control path with a given set of constraints. This makes use of Rosen's gradient projection method.[3] The upshot of this is that such optimum control leads to solutions for the optimal drug rates which can be fitted within exponentially decaying envelopes centered about the steady state input values. Thus equation 4 is still used, but now the input values X_i are held to X_i where X_i is limited maximally to the maximal drug rate allowed or the value dictated by the upper border to the exponential envelope whichever is least and minimally to the minimal drug rate allowed or the value dictated by the lower border of the exponential envelope whichever is greatest. Such control we have come to call phase III control.

In mathematical representation:

$$X_i = \begin{cases} UB_i, & X_i > UB_i \\ X_i & LB_i < X_i < UB_i \\ LB_i, & X_i < LB_i \end{cases}$$

$$\text{where } X_i = \frac{r_i - \sum_{K=2}^{N} W_k X_{i-k+1}}{W_1}$$

$$UB_i = \min [d_i, UC_i]$$
$$LB_i = \max [c_i, Lc_i]$$
$$UC_i = X_{ss} + v \exp [-\psi(i-j)]$$
$$LC_i = X_{ss} - v \exp [-\psi(i-j)]$$

where

d_i = upper limit of I.V. rate (input)
c_i = lower limit of I.V. rate (input)
$\psi = 1/NL \ln v/\delta$

where

L = time between weights
N = number of weights
μ $\Delta \max | X_{ss} - X_i |$ allowed at time i = j
δ $\Delta \max | X_{ss} - X_i |$ allowed at time i = T

RESULTS

A run is begun under open loop conditions. That is, the physician commands the drug rate. However, the model "observes" these activities.

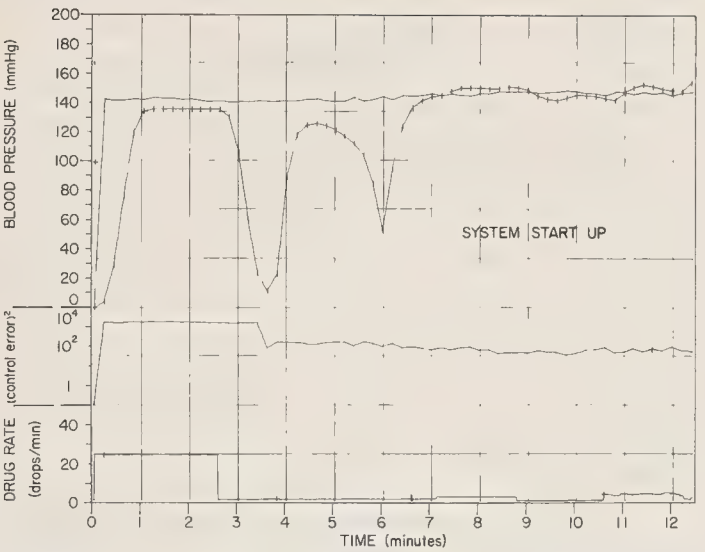


FIGURE 6.—A typical run start up. The predicted blood pressure (+++) converges on the actual blood pressure (———).

Figure 6 shows a typical example of such a start up. Note how the predicted blood pressure value converges on the actual value. Figure 7 is later on after the model has converged and the blood pressure is being held at an average of 160 mm Hg using a vasopressor (Levophed) in the I.V. This is higher than the dog's normal blood pressure and was used to allow the model to converge. At about two minutes the affect of Arfonad becomes apparent. While still on manual control the average blood pressure command is set for 100 mm Hg and the I.V. rate in-

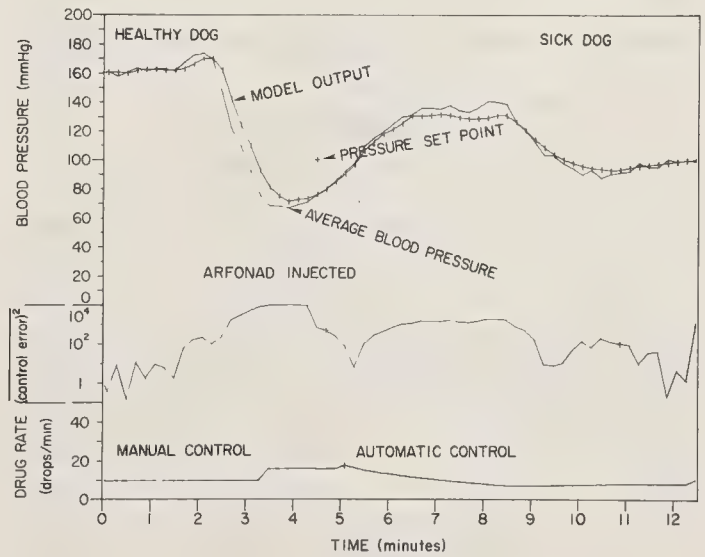


FIGURE 7.—The initiation of automatic control and the system's response to the administration of Arfonad. This is phase I control.

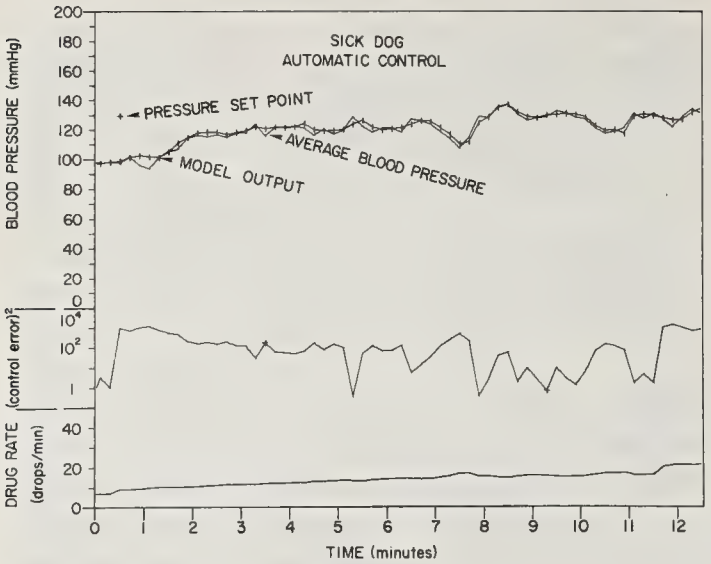


FIGURE 8.—The response to command changes in blood pressure with phase I control.

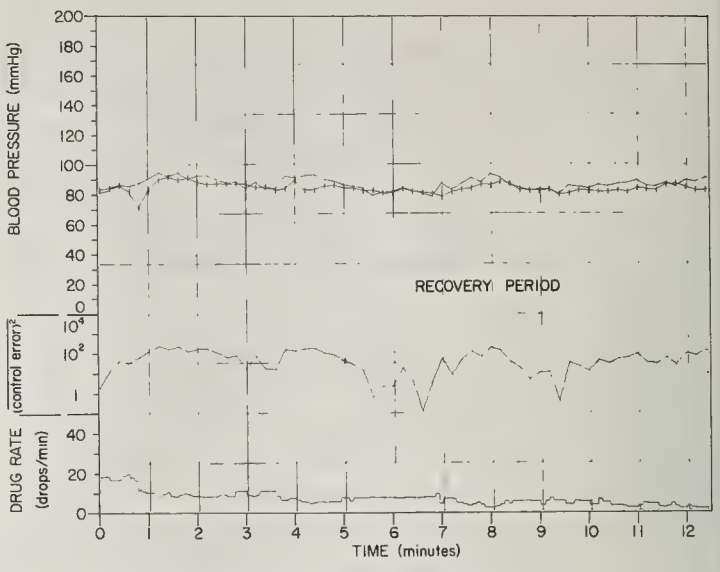


FIGURE 10.—The weaning of the vasopressor I. V. during the recovery period.

creased. At five minutes the loop is closed. To be noted is how well the model's output tracks the blood pressure. Figures 8 and 9 are direct continuations of the previous figure. Figure 10 is the weaning period where the controlled discontinuance of the drug is the point of interest.

Note that the blood pressure set is changed from time to time and how the blood pressure responds. This is phase I control; that is, we are using steady state control. This accounts for the rather slow change in blood pressure after a command change.

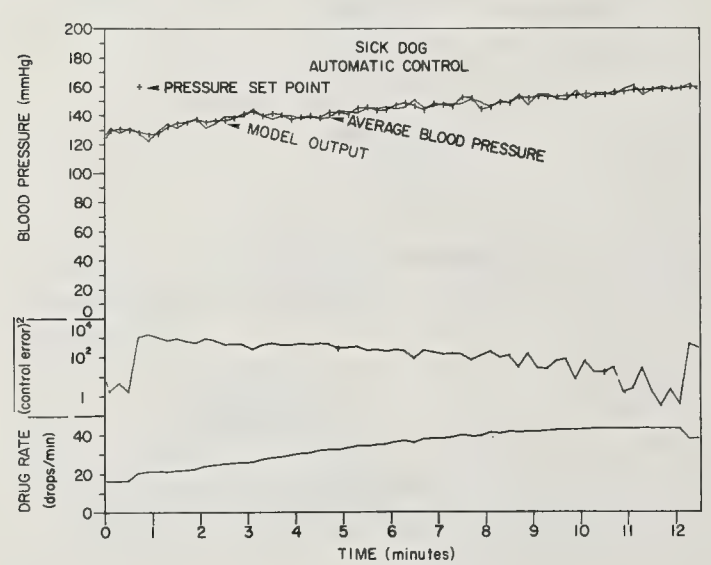


FIGURE 9.—The response to command changes in blood pressure with phase I control.

Figures 11 through 14 are examples of similar maneuvers except now phase III control is being used. Note the rapidity of response to a given command change or other perturbation.

FUTURE WORK

We are currently attempting to implement phase IV control where optimal solutions will be determined for all constraints. In addition, we are working on asymetrical control. The vasopressors we have dealt with to date cause quite

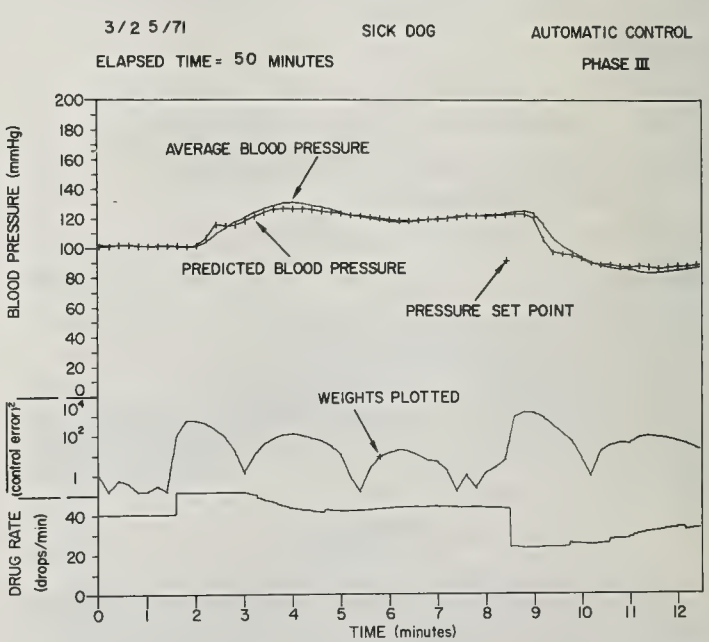


FIGURE 11.—An example of phase III control.

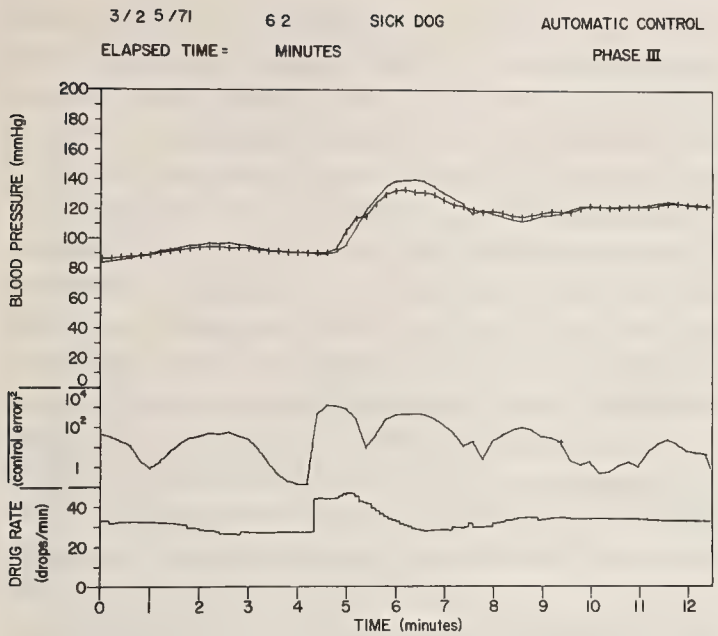


FIGURE 12.—An example of phase III control.

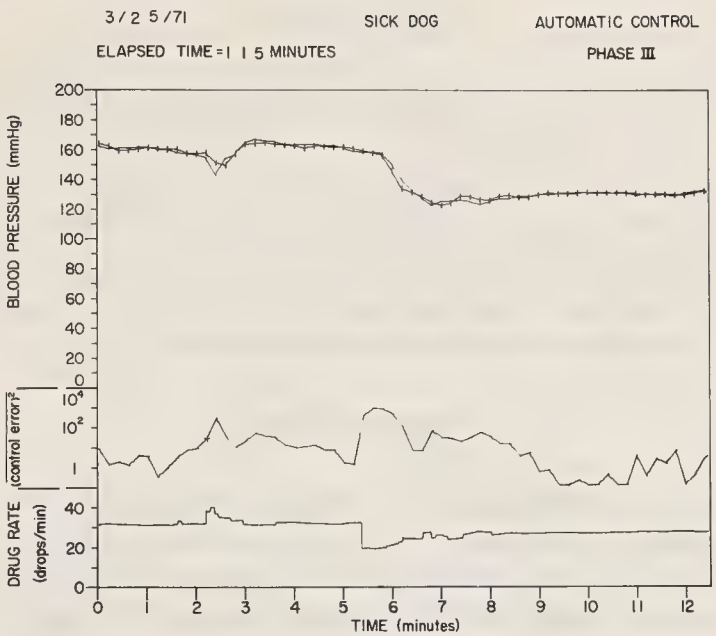


FIGURE 14.—An example of phase III control.

symetrical responses. That is, the dynamics of adding the drug are quite similar to those seen while removing the drug. The present approach would lend itself well to the control of hypertensive crises except that the ganglionic blockers etc. appear to cause quite asymetrical responses. Thus, an asymetrical model is required. Asymetrical modeling requires only that we use two models, one for each direction. We are also investigating multiparameter control as required for shock control. In addition to the clinical entities noted above we are also examining the control of cardiac arrhythmias.

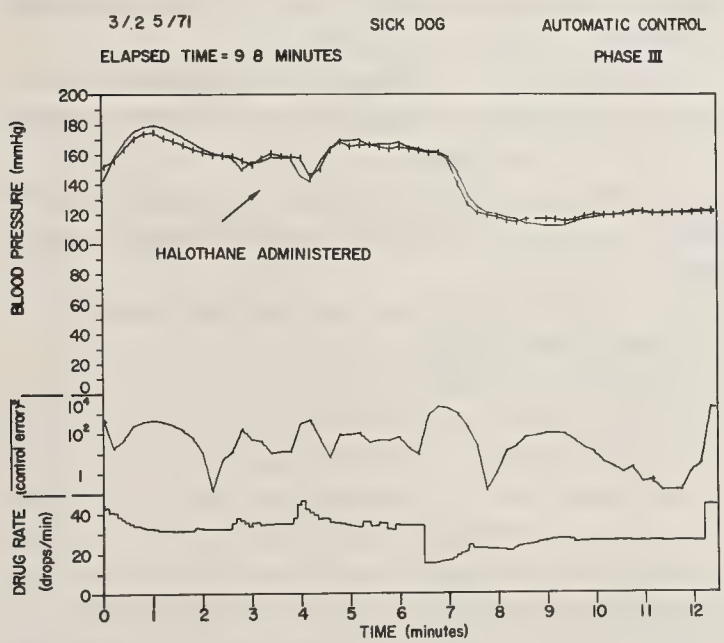


FIGURE 13.—An example of phase III control.

Another aspect of the whole question of control that we are studying is what we call concurrence control. To make such systems more acceptable to physicians, we are studying the feasibility of allowing them to concur with all decisions made by the controller. Thus the controller will have to get the physician's permission to make any change in drug rate beyond preset bounds. Obviously no such system will ever be optimum, but because of its adaptive qualities, it is hoped such systems will function adequately despite the delays caused by the physician.

SUMMARY

We have been studying a relatively simple therapeutic control problem, the support of hypotension in dogs with a vasopressor. Such a procedure is attractive as a starting point because one can easily measure and effect blood pressure. Also, such a control problem has the characteristics common to most therapeutic control situations i.e., a nonexplicit time variable delay and response.

ACKNOWLEDGMENTS

This paper is the result of the Ph.D. work of Dr. C. M. Schade done under Dr. Widrow with the assistance of Dr. Thompson.

REFERENCES

1. Widrow, B., and Hoff, M. E. Adaptive Switching Circuits. IRE Wescon Convention Record, Part 4, pp. 96–104, 1960.
2. Widrow, B. Adaptive Sampled-Data Systems. Proceedings, First International Congress IFAC, Moscow, pp. 406–411, 1960.
3. Rosen, J. B. The Gradient Projection Method for Nonlinear Programming, Part I—Linear Constraints. J. Soc. Indus. App. Math. 8, 1960.

DISCUSSION

P. G. Katona, Case Western Reserve University, Cleveland: I would be interested to find out why you decided on the adaptive control model rather than pattern recognition techniques? And especially, it seems to me that correlation analysis would be useful. These can be usually characterized by expotential type of functions. I assume that you would just do what these analyses indicate. You might be able to get away with a much simpler technique.

Dr. Thompson: You are right. In fact, we could probably do this by adapting only two parameters—delay and gain. Now, in fact, we approached the problem that way initially and then I built a small special purpose computer to do it. There's one hooker to that. If we lived in a world without noise that would work fine, but unfortunately we live with noise. Blood pressures fluctuate. They don't appear as perfectly orderly functions. Therefore, we have to have some way to detect when a response is over. It's a simple response and, yes, it's an expotential, but you have to know when it's over to make a measurement to decide if you got the response you wanted. You can use correlation techniques and pattern recognition techniques but then you're getting into the need for a computer again. I mean, now we're talking about memory and we're talking about a lot of complications. We had the technique described here already available to us, and it gets around having to do the more complicated pattern recognition problems.

Our Phase I control is simply a delay and a first order expotential model plus a way to find where you are in a field of noise. Now, there's more to it though. If you try to do Phase III control, that is where you try to move states around optimally, you are dealing with a more complicated problem, and we could ask how far one need go in the direction of optimum control. At present, I don't know. As a researcher, I think it's exciting, but as a practical physician, I'm not sure whether we need optimum control. But if you are going to try to approach optimum control with the idea to get the pertinent states to follow your input commands as closely as possible, then you probably have to know a little bit more about the system's dynamics than can be represented by only two data points. I didn't say why we actually used 21 data points. They happen to be five seconds apart and it takes 21 x 5 on the average for this animal, the dog, to get through his response in the worse case. So we're trying to cover the worst case so we can model it. I think I've answered all your questions. If not, I'd be happy to take another whack at it.

W. J. Sacco, Edgewood Arsenal, Maryland: I would like to try to translate what you're saying into the language that I know for the general problem that you solved. Now, did you have anything else besides the delay that you were trying to identify as you were going along? Were you trying to identify other parameters besides delay?

Dr. Thompson: And gain.

Dr. Sacco: And gain, right. Okay, now, the delay—you considered that to be a random variable constantly changing?

Dr. Thompson: Delay is capable of constant change. In fact, it changes very slowly over a long period of time.

Dr. Sacco: So, you were doing systems identification along with the control problem throughout?

Dr. Thompson: Yes, our model is in fact a systems identifier.

Dr. Sacco: Constantly?

Dr. Thompson: Constantly! I didn't go into the mathematics. I think it was my fourth slide (see equation 1), where you have all these weights and you're comparing the output of the model with the output of the plant. What you basically do is say, "I've got a weight vector. I'm going to increment that weight vector by delta weight vector," which says I'm going to

multiply the error times a fenagle factor, which we call the convergence factor, times the present input vector, divided by the correlation of this input vector with all of the previous input vectors. What you're saying is: "See if this input is very unique; if it is, we're going to use a lot of it. If it's not, we're going to use a smidge of it." We're going to change our weights now by some percentage of the total change we need. We don't adapt in one step. We do not take our weights from some error to no error in one step. If you do, you get into instabilities. The fact is we used a convergence factor of 0.4 which takes us about three to four passes through the filter before we are down to where we'd like to be. This provides stability. In general, it gives us no big problem. I'd be happy to give you the equations if you'd like to see them.

The big problem, as you may recognize is taking the inverse of this model (see equation 4). In fact, you have a problem because the denominator of this particular equation happens to be weight number one, which may be zero, so that you're in trouble right off the bat. In fact, we go through our model and find out which is the first weight that is out of the noise. We use this weight as weight one. We remember how many weights we took off and that's our delay. Then we take all the dynamics and put them back on. You must also understand that we are dreamers. For blood pressure control this model is probably a cannon shooting a fly, we hope some day to go to more complicated things. We can visualize, for example, using a system like this where one can generate an Nth dimensional space and do multiparameter control.

For example, maybe you don't just want to give this patient a volume expander and then an inotropic agent or vice versa. To get from point A to point B, you may have to follow a rather circuitous course without going into some forbidden zones. You see, we're really trying to think ahead. So this model is not just to resolve this one problem, but we hope that it will give us the background to tackle more erudite problems later on.

CHAIRMAN: I think this is a very exciting paper and I have another question I'd like to ask you. In starting a new dog, or a new case, do you start with what you learned from the previous one or do you start from scratch again? It seems to me you could start with some sort of general average of where you were.

DR. THOMPSON: Yes, that was what I was—in my befuddled way—trying to point out. The model that we started out with was shown in the slide of the weights converging which had a peak, this is also the impulse response. That began with the model's weights for "our average dog." Now admittedly it was a poor one for this particular dog, but in general it's very close to where we're going, so that we don't have to change things very far, which cuts down our initial learning period. We could actually start anywhere.

K. B. LARSON, Washington University, St. Louis: Have you attempted to devise any biological models of the parasympathetic and sympathetic nervous systems and their different input-output response such that your adaptive process could shed light on?

DR. THOMPSON: No, we in fact avoided that question, because I think that's an entirely different question. There is one thing, along the medical line that we, of course, thought about and it was inherent in what I said just a moment ago. Maybe these models can give us some clues in pharmcodynamics, in the dynamics of drugs used in a multiparameter sense. That's biological modeling, if you wish. But trying to find out what the parasympathetic or sympathetic nervous systems' roles are and so forth, no. We've made no attempt to do this.

REAL-TIME, TIME-SHARING COMPUTERS IN THE ANIMAL LABORATORY

W. S. Topham*

The classical approach to analyzing physiological data obtained from animal experiments has been to record the analog waveforms on an oscillographic recorder. This direct observation coupled with tedious manual measurement have given insight into physiological phenomena. The use of digital computers has allowed the reduction of data to be performed more efficiently as well as providing a convenient storage medium from which the data easily can be retrieved and analyzed at a future date. Early in the establishment of the Utah Biomedical Test Laboratory in Salt Lake City, and after a study of data acquisition was conducted, a sixteen-bit general purpose computer was chosen to serve as the heart of the data acquisition system. The advantages of using the computer (i.e., automatic data collection and reduction; immediate display of calculated values; storage of calculated data for easy retrieval, future analysis and automatic report generation) were used as guides in developing the data system. Several users time-share the computer in real-time and the main control of high priority foreground is through remote terminals. A program is loaded into memory and a message is displayed indicating the options and operation of the program. The desired option is selected. This concept makes many programs available for on-line analysis to users in the operating rooms, intensive care area, and catheterization laboratory. Some of the "user" programs which have been written and are currently under final test provide for analysis of indicator dilution curves, measurement of oxygen saturation, evaluation of any physiological pressure and analysis of any physiological flow. A data review program allows a user to review at any time any data that has been collected on a given animal. An automatic monitor is being written which will sample up to eight channels of data from any animal every fifteen minutes. This program is controlled by a remote terminal located in the monitor mezzanine where there is visual and audio contact with all operating rooms and the animal intensive care area. If the computer detects an alarm condition, an indicator alerts the terminal operator, and the necessary corrective action can be taken.

INTRODUCTION

Physiological data have been analyzed classically by the the use of analog recording devices. From the use of a smoked drum to the present sophisticated multi-channel pressurized ink systems and optical systems, the analog physiological waveform has provided assistance in the understanding of the complex functioning of the living organism. These methods are important and some will continue to be used, but they have limitations. While they provide observations of trends, variation in maximums and minimums, waveform variation and timing relationships, it is difficult to obtain areas under curves, extract mean values, and obtain mathematical relationships of the variables. Part of this problem has been solved by the use of the analog computer or special electronic preprocessing devices which generally use the heart of the analog computer, the operational amplifier.

Calculations have been done using the analog computer in the real-time mode, [1,2] but the results must still be stored using an oscillographic recorder. To compare the data obtained at various times during the experiment, a rather tedious search through rolls of oscillographic data need be undertaken, and to arrange the data with statistical significance requires laborious hand digitization. These techniques cause significant delays in obtaining the desired information. However, if the analog signals are directed into an A-D converter of a digital computer, the calculated values are obtained, stored, and are easily retrievable. With the computer operating on-line, the calculated values are immediately available to the researcher; and during the experimental run, the results are displayed. This provides a feedback which keeps the researcher continuously informed with analyzed data while the experiment is in progress and often assists in guiding the direction of the experiment so that more significant data may be obtained.

The digital computer can be used to automati-

* Director of Biophysical Services, Utah Biomedical Test Laboratory, Salt Lake City, Utah.

cally monitor an experiment by sampling, analyzing, and storing data at pre-set intervals, or it may monitor on an interrupt basis. The interrupt can occur frequently, such as with each "R" wave of the ECG, or infrequently, only when a variable exceeds desired limits. Any of this automatically monitored information can be made available for review almost instantly by using the computer data file for storage.

At the Utah Biomedical Test Laboratory (UBTL) in Salt Lake City, the problem of data acquisition and analysis is significant because of the large amounts of data that will be generated. Early in the planning of the Laboratory, it was determined that a digital computer would serve as the center of the data system. The size, operation, and capability of the computer were studied thoroughly. It was soon evident that some capability would have to be sacrificed because of the limited funds budgeted for the purchase of the computer. The main function of the computer is data acquisition and primary data reduction. A general purpose, sixteen-bit computer, XDS Sigma 3, with good interrupt capability and excellent input/output (I/O) handling was selected. This machine has 32K of memory and standard peripherals, 400 wpm card reader, 200 lpm line printer, nine-track magnetic tape and 1.5M word Ready Access Disk (fixed head). The on-line data handling capability is presently provided by a 64-channel multiplexer and A-D converter, and extensive digital I/O interfaces.

DATA SYSTEM

The Test Laboratory building consists of three stories with the first floor containing the animal holding areas. The second floor is comprised of surgery, the catheterization laboratory, and an intensive care area with their supporting laboratories and supply rooms; and the third floor contains offices and engineering laboratory space. Located in the center of the building is a monitor mezzanine. From this mezzanine, which is elevated above the second floor, there is visual contact with the four operating rooms and the large intensive care unit (ICU). From here, it is possible to observe surgery as well as the activities of the animals in the ICU. There is audio contact between the mezzanine and all animal areas. Figure 1 is a schematic floor plan showing all the animal sites, including the animal holding rooms on the first floor.

At each animal site in the Laboratory, an animal monitor console can be located. Internal signal wiring provides for one monitor console in each operating room and the catherization laboratory. Forty consoles can be placed in the ICU, and there is wiring for one console in each holding room, although three to four animals can be placed in each room. The monitor console is built in modular fashion and can amplify and condition up to sixteen analog variables at any one time. It also provides switching capability so that any eight variables can be sent to the computer for analysis, and any eight variables can be sent to the monitor mezzanine for observation and recording. The console provides digital readout for a limited number of variables and also some preprocessing of the signals so that a limited number of mean, systolic and diastolic pressures can be displayed at the animal site. There are also alarm circuits connected to each analog amplifier-conditioner, and preset alarm levels will generate acute alarms at each site. These alarms are generated when life-threatening conditions exist, such as cardiac arrest or severe respiratory distress.

Under development in the monitor mezzanine is a master monitor console (MMC), shown in Figure 2. This console is physically located for convenient observation of the animals in the ICU. It contains a patient selector system which gives indication whenever an acute alarm is generated at any animal site and permits the selection of eight signals from any animal for display. The MMC also contains a remote computer terminal which is used to control the automatic monitoring of the animals at any location in the Laboratory. There will be an indicator panel associated with this terminal which has a set of three lighted indicators for each animal site. One will light when the animal data is being sampled; another, when routine medication should be given; and the third is both a visual and audio indicator that is turned on when the computer detects that calculated physiological variables have exceeded preset limits. Whenever any alarm condition exists, either the acute

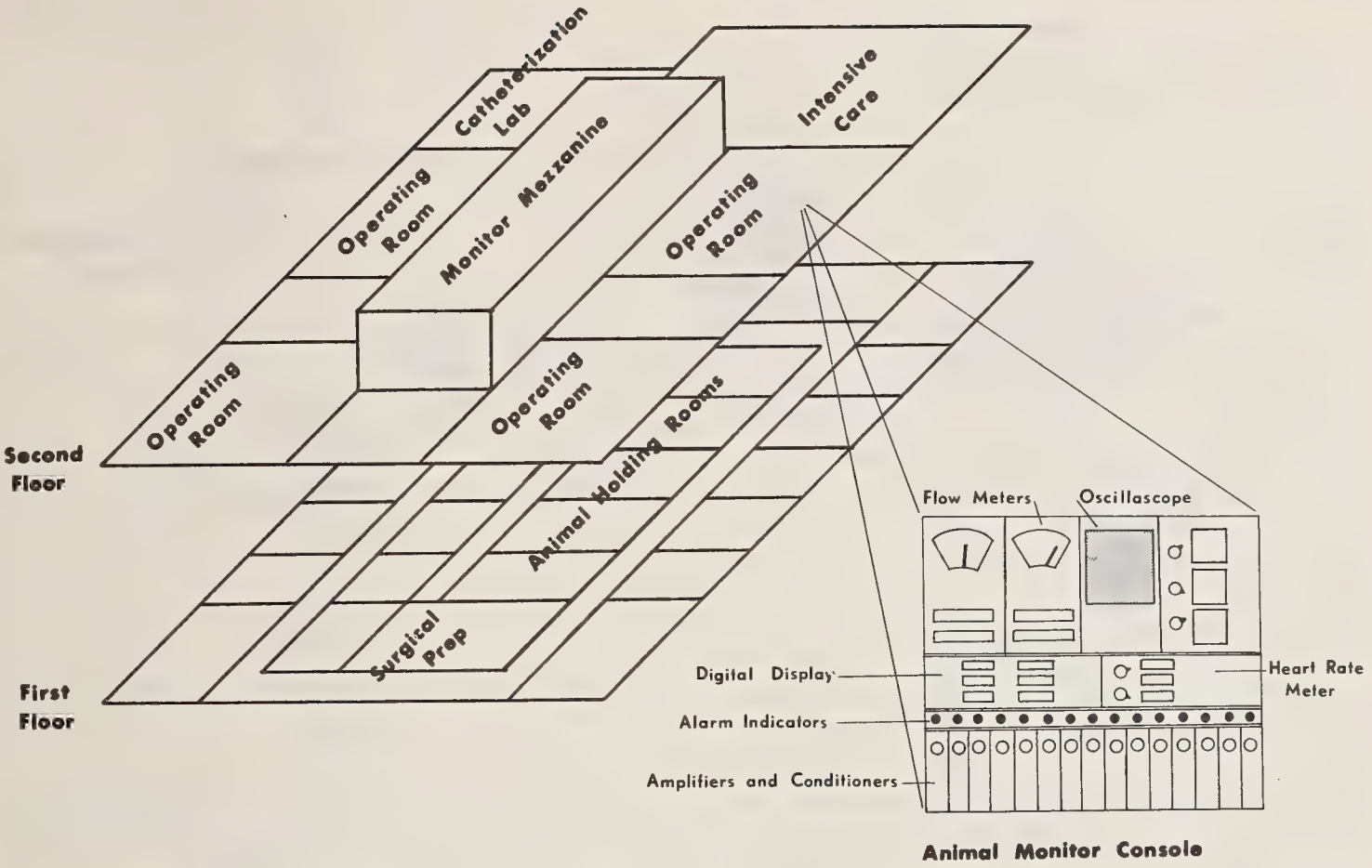


FIGURE 1.—A schematic floor plan showing the locations of animal sites in the Artificial Heart Test Facility. Animal monitor consoles for acquiring and conditioning analog data can be located at any animal site as described in the text.

alarm or the computer alarm, it is possible to immediately switch the monitored variables from that animal to any one of the recording devices (analog tape recorder, ink oscillographic recorder) for recording and/or observation.

From the monitor mezzanine, it is possible to determine the physiological state of any animal. Any data which has been collected by the computer can be retrieved and displayed on the terminal; current computer measurements can be made and immediately displayed; the analog signals coming to the computer and monitor mezzanine can be observed on an oscilloscope and if desired, can be recorded on the ink recorder; and any prerecorded analog signals can be viewed.

When animals are in the intensive care unit, the main monitor console will be manned twenty-four hours a day. Using this technique, one individual can monitor many animals simultaneously, and only when alarms or difficulties occur is it necessary to involve other personnel.

It is the purpose of this data acquisition scheme to eliminate continually running analog tapes or oscillographic recording devices, and to eliminate piling up formidable mountains of records for eventual analysis, which often never gets done. The main monitoring is done by the computer at intervals of fifteen minutes, and it is anticipated that the alarms can be set accurately enough that sufficient data can be obtained to determine the cause of the alarming situation. If there is a need for more frequent measurement, it can be initiated manually. Only in exceptional cases when it is necessary to observe the analog waveforms will the analog recorders be turned on.

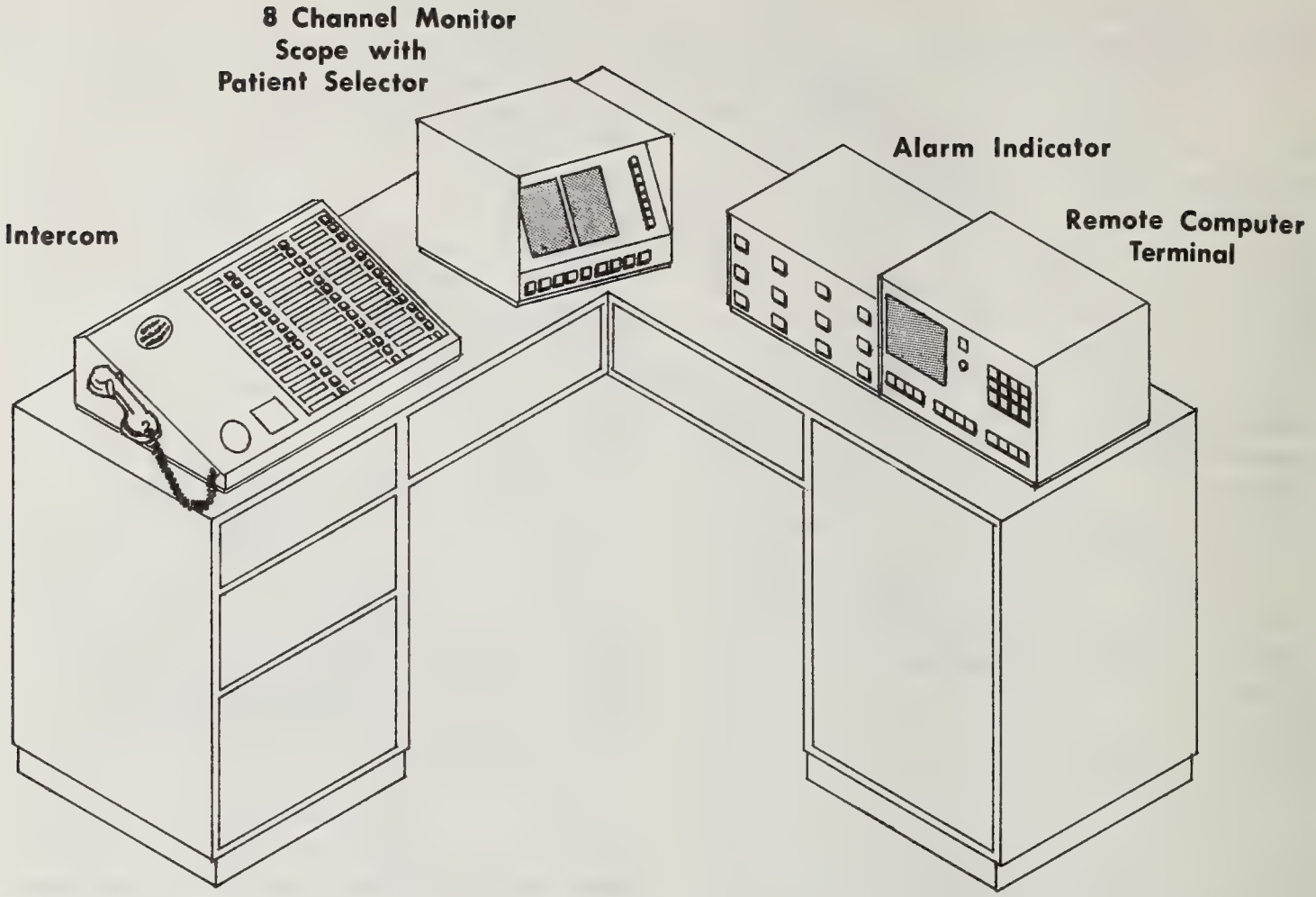


FIGURE 2.—Functional drawing of the Master Monitor Console located in the monitor mezzanine. Automatic monitoring of all animals will be controlled from this console.

COMPUTER SYSTEM

The computer is important for performing the automatic monitoring of the animals; but, in addition, it must be able to perform other functions. Therefore, a time-sharing system was set up.

The real-time, time-sharing system is based on the MEDLAB System developed by Warner and associates[3,4,5] at the Latter-Day Saints' Hospital in Salt Lake City. There are significant differences between these systems, however, because the computer that is used by UBTL is a sixteen-bit computer, has less instruction capability, and also has a rather sophisticated I/O mechanism which allows I/O to be carried out independent of the central processing unit (CPU) operation. Also, the AHTF basic monitoring requirements are much more variable since each device tested will require a different set of parameters to be analyzed.

The core memory of the computer is broken down as shown in Figure 3. At lower core is the real-time batch monitor system (RBM) which is provided by the computer manufacturer. Next to this is the Utah Time-Sharing Executive (UTE) which is the executive that provides us with the real-time, time-sharing function and is the subject of this discussion. A section is devoted to the AUTOMON program, which carries out the automatic monitoring of the animals. There is a "User Area," and at the present time, this area is divided into three 2K segments. All of these areas are located in the foreground section of memory and are thus under the control of the real-time interrupts.

While the computer has a priority interrupt scheme, it is used to little advantage because

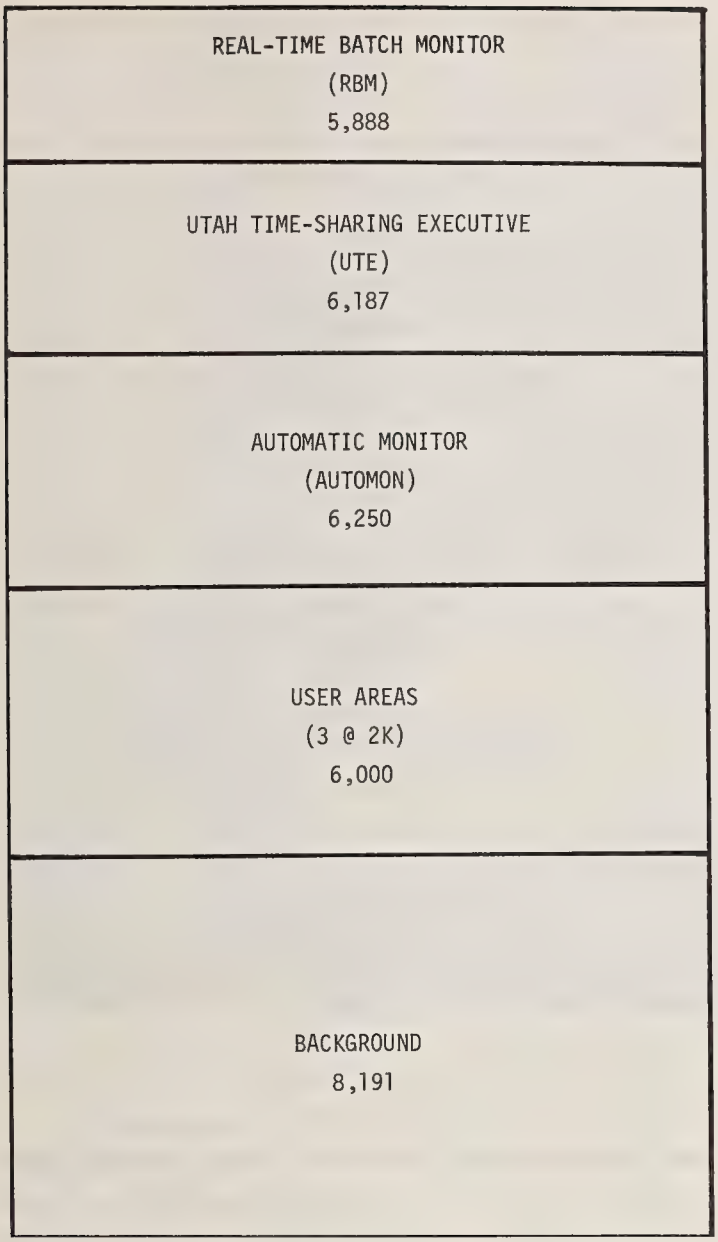


Figure 3.—Core map of XDS Sigma 3 computer operating in a real-time, time-sharing mode. UTE was written to provide this capability and is described in the text.

each remote terminal located in the Facility is connected to one of these interrupts, and each terminal should have equal priority. The executive does use the higher level interrupts to guarantee that it can perform its bookkeeping without being interrupted at critical times. However, when an interrupt comes from a computer terminal, the time-sharing executive within a few hundred microseconds places the operation of that terminal at low priority, allowing any other terminal to interrupt at any time. The interrupt which controls the data sampling through the DATAIN subroutine is connected to a high-order interrupt and guarantees that when it is necessary to take data samples, they are taken at the correct time, without delay.

The last section of core is devoted to background activities and provides an off-line operation for the computer. This includes such activities as compilation of programs, extensive searches for data, hard-copy reproduction of data, as well as any other off-line batch jobs that can be accomplished in the background area available.

Time-sharing in the foreground area is carried on mainly under the control of the UTE, and if there are no activities to be accomplished in the foreground, then control is relinquished to background. It is primarily controlled by RBM. At the present time, because of limited core space, foreground is time-shared between three users and the AUTOMON activities, simultaneously. However, the executive has been written to time-share at least eight users, and this can also be expanded depending on the computation time available, as well as more core memory.

Executive Subroutines

Some of the important subroutines used by UTE to accomplish its data handling are the following:

SCOPEWRT: This subroutine is used to carry out communications between the remote terminal and the computer. It writes the desired message on the screen of the terminal, plots any data that is desired on the terminal as governed by the 512 by 512 matrix capability of the terminal, and reads the information generated by the terminal.

DATAIN and CLOCKINT: These subroutines are used together to provide the analog data sampling function. Any user can sample up to eight channels of analog data. It is possible with these routines to sample those eight channels at any four different sample rates and return to the user program for computation after any given number of samples have been taken. At the present time, there are sixty-four analog channels connected to the computer, and when any sampling is done, all sixty-four chan-

nels are sampled. It is then the responsibility of the CLKINT routine to sort out the information of only those channels requested by the users. Sampling is done at a minimum period of five msec, 200 samples per second, and samples can be obtained at any interval which is a multiple of the five msec period up to periods of several seconds.

DISKIO: This subroutine is responsible for handling the transfer of all data between the user programs and the fixed-head disk. The data is stored under a file structure using four different sort keys: Patient Number, Device Number (the number of the device under test), the Program Number of the program which generated the data, and the Time at which the data was obtained. The disk is divided into pages of 180 words each. DISKIO is responsible for sorting and writing the data on the correct pages as well as reading the correct data when any request is made for information based on the four sort keys. If data from a new patient is to be added to the disk, this subroutine finds those pages which are available and stores the data.

Since the datafile is maintained by chaining pages together according to the two sort keys, Patient Number and Device Number, it is the responsibility of the subroutine to maintain this chaining. Also, if a patient is to be deleted, DISKIO stores the necessary information for bookkeeping and returns the pages used by that patient to the "Page Available" pool. Variable length records can be stored, but the user program must specify the length of the record which it is generating. It can be seen that since DISKIO is the only way in which a user program can write data on the disk, it is totally responsible for the maintenance of the datafile and will greatly reduce the probability of data being destroyed because of incorrect information which might come from the user programs.

Remote Terminals

To use the computer system, remote terminals shown in Figure 4 were selected and are located at various sites throughout the Facility. This terminal uses a storage tube for its display and was selected because it has a 512 by 512-point graphic capability and also can display Alpha-numeric data. It is also relatively low in cost as compared to other terminals with the same graphic capability. The terminal interacts with the computer by using two octal switches and a 12-key keyboard. Located between the two octal switches are two call buttons, one for each octal switch, an indicator light which illuminates when the station has been recognized by the computer and is active, and a manual button for erasing the oscilloscope. Another feature which is available on the terminal is that of being able to display analog signals. Three signals can be connected to the back of the terminal, and by pressing the analog display button, the three signals are displayed simultaneously. The sweep rate can also be varied. If information comes from the computer during the period in which the analog signals are being displayed, the display is automatically terminated and the information from the computer is written on the oscilloscope.

PROGRAMS

When a program is to be run from a remote terminal, the program number is dialed into the octal switch and the call button depressed. This interrupts the computer, which then reads the octal switch, determines which program is to be used, retrieves the program from the disk, and stores it in one of the available user areas. Control is then given to the program and a message is written on the oscilloscope indicating which options are available in the program and how to proceed with its operation.

Dye Dilution Curve

One of the user programs which is currently available on the system does analysis of indicator dilution curves. It is described in some detail to illustrate how the programs are designed. Using the Stewart-Hamilton method, this program calculates cardiac output and other parameters which are of hemodynamic importance, such as, appearance time, build-up time, mean circulation time, and central blood volume. If this were the program called, the displays that would occur on the oscilloscope are shown in Figure 5. The first display is the iden-



FIGURE 4.—The remote terminal (Beehive Medical Electronics α-21) used in the computer system. A storage tube display gives 512 by 412 point graphic capability.

tification message. The correct patient and device number are entered and verified, as is the number of the analog channel transmitting data. To continue, button '3' is pushed. This stores the entered values on the disk, and the main message is displayed on the oscilloscope.

If this is the first time the dye curve is run, then calibrations are taken. Black and saline values give the range of the cuvette. Black is the voltage from the cuvette with no light being detected and is obtained by an electrical setting. The saline value is obtained by withdrawing saline through the cuvette and sampling the output voltage. Blood is then drawn through the cuvette and zero dye calibration is taken. Dye is added to the sample to provide a concentration of 12.5 mg/l and reinfused through the cuvette while the 12.5 mg calibration value is sampled. If these values are satisfactory, then '4' is pushed and the calibration values are stored on the disk so they can be recalled and used for future dye curve analysis.

It is then necessary to enter the number of seconds the dye curve is to be sampled. This is usually about forty seconds. Whenever a value is to be entered, a double asterisk will appear by the number which has been pressed and will remain until the value is entered and the 'SEND' button pushed to enter that value into the program. The amount of dye to be injected is entered depending on the size of the animal. This is usually two ccs of dye when a concentration of 12.5 mg/l is used. If all the values are correct and verified, then arterial blood is withdrawn through the cuvette oximeter, and at the moment the dye is injected into the venous system, the number '7' is pressed. The program then samples the output of the cuvette for a period of time specified as the dye circulates through the animal and the cuvette.

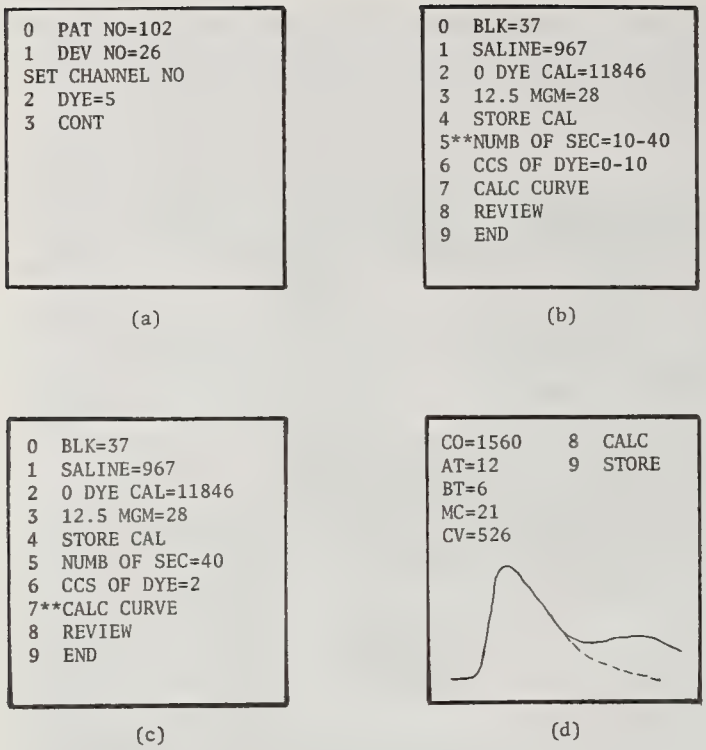


FIGURE 5.—Messages displayed on the remote terminal when running the Dye Curve program. (a) Identification message. (b) Calibrations have been entered; the number of seconds the curve is to be sampled is to be keyed in. (c) The curve is being sampled for the forty-second period. (d) The results are displayed and may be stored by pressing '9' on the keyboard.

At the end of the sample period, an extrapolation is performed on the return limb of the dye curve to return the concentration to zero and remove the error introduced by the recirculation of dye. The extrapolation is exponential and the value of the exponent is determined by a constant which is the logarithmic slope of the descending limb of the curve prior to any recirculation. When the dye curve analysis is complete, the sampled dye curve and the extrapolated dye curve are displayed to be sure that the extrapolation is correct. The calculated values are also displayed at this time and may be stored. Subsequent dilution curves can be run using all the constants which were previously entered. Button '8' is merely pressed at the moment of the next dye injection. As the data is stored, the time-of-day clock is sampled and the value is stored with the data.

Oxygen Saturation

This program calculates and displays the oxygen saturation as percent saturation from the signals which come from the infrared (IR) and red (R) photosensitive devices in a cuvette oximeter. Since this method of measuring oxygen saturation uses the transmission of light, the relationships are logarithmic, and oxygen saturation is calculated by obtaining the ratio of the logs of the intensity of light detected by the IR and R cells. This program uses the same instrumentation as is used for recording dye dilution curves. It provides for frequent on-line readout of oxygen saturation without having to transfer the blood sample to a laboratory instrument for measurement.

Pressure

This program calculates the maximum, the mean, and the minimum of any physiological pressure. Because it is difficult to do a pattern recognition on many of the pressure waveforms, a trigger is used to indicate the beginning and end of each heart cycle. This trigger is a pulse which is generated with each "R" wave of the ECG. The maximum and minimum during each cycle is determined and the mean is found over the same period. The values for eight beats are obtained and then averaged, and the averaged result is displayed on the oscilloscope for observation. Since the time of day is recorded with each averaged value and the measured pressure is identified with a number code, any number of pressure measurements can be made and stored for later trend and/or statistical analysis. The tedious process of manual recording and plotting pressure values from analog preprocessors and digital displays or measuring values from oscillographic recordings is eliminated.

Flow

This program also uses the trigger pulse and can calculate the maximum, minimum and mean of any physiological flow. It also has the capability to calculate stroke volume and thus cardiac output from a flowmeter which is placed on the ascending aorta. While many flowmeters give a calibrated mean flow reading, the use of

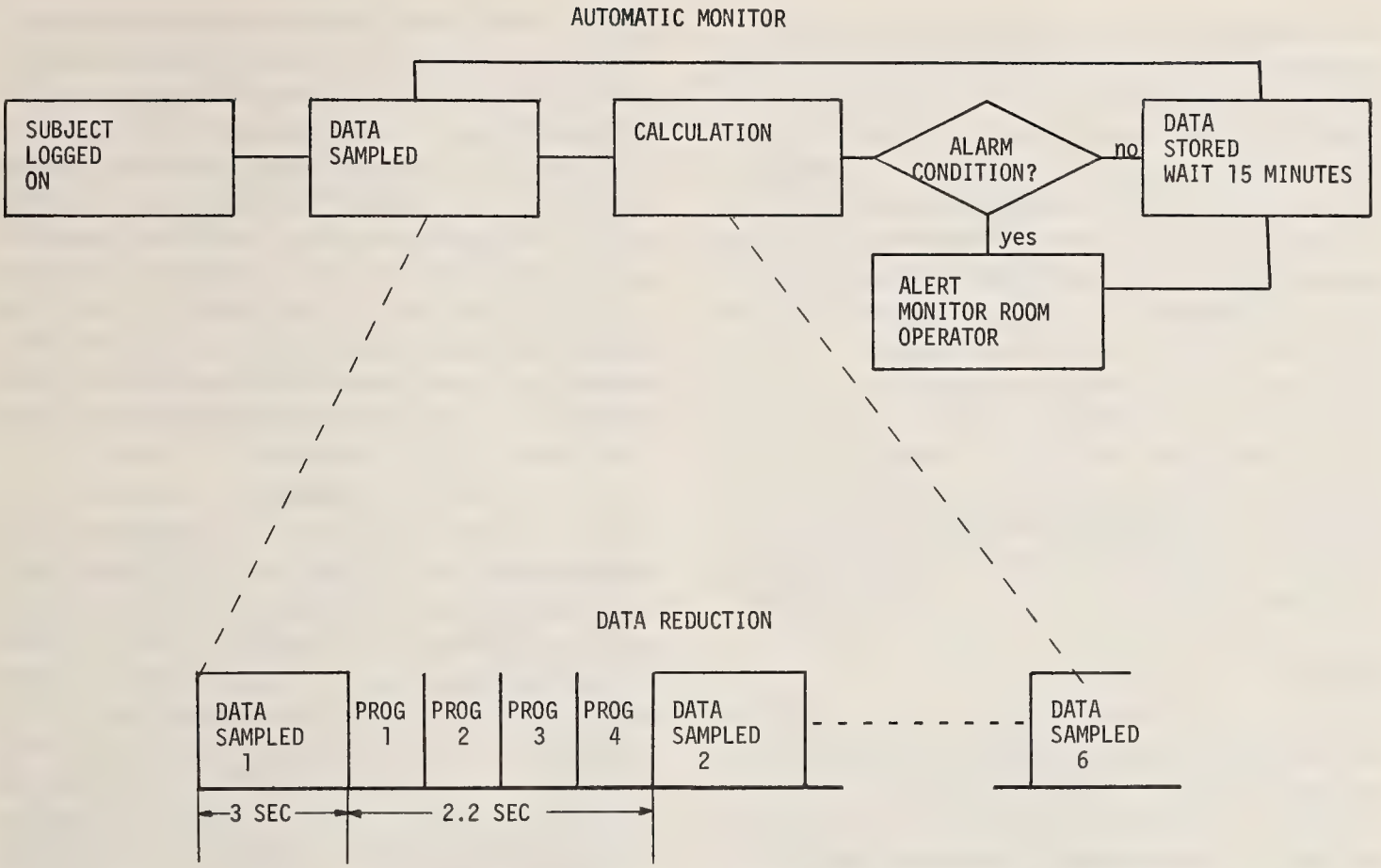


FIGURE 6.—Functional diagram showing the operation of AUTOMON, the program which will automatically monitor the animals.

the flow program gives a maximum and minimum reading simultaneously and stores all values for future analysis. No digitization of oscillographic records is necessary.

Pressure Pulse

This program, using the method of Warner,[6] calculates stroke volume from the central aortic pressure. With this information, it is possible to calculate cardiac output. The mean pressure is obtained as well as the systolic and diastolic pressure, and peripheral resistance can be calculated. Other variables which also are available in this program are stroke work, change of pressure with respect to time, dP/dT, and systolic duration. This program does not use a trigger signal, but does a pattern recognition on the central arterial pressure waveform, and by finding the onset of systole, the peak pressure, the dicrotic notch, and the end of diastole, the above calculations are made. It would take many hours of calculation to obtain the information from the aortic pressure that the computer, using this program, is able to display within seconds.

On-Line Data Review

A program which functions in a different manner from the above programs is the On-Line Data Review, which is operated by use of the remote terminals. This program allows an experimenter at any time to review the data which has been obtained on any animal. The animal and device number are entered, and if all data is to be reviewed, then a display is called and the most recent information associated with that animal is displayed. The review then can proceed in a chronological manner, and by merely pressing a button, the data is displayed page by page or one measurement at a time, depending on the option selected. At any time, the operator can move ahead or backwards at hour intervals and review the data, or he can select a given time to the nearest hour when he would

like to look at data, and this information will be displayed. When several programs have been used to acquire the data on a given patient, it is necessary to limit the amount of data to be reviewed. For instance, if pressure data and oxygen saturation data were obtained, it may be desirable to look at the time course of the oxygen saturation without the pressure data being displayed. This option is available by specifying the program number of the desired data.

It should be remembered that each of the above programs occupies one of the user areas on the computer, and at the present time, any three of these programs can be running simultaneously on a time-shared basis. In addition, automatic monitoring of several animals can be continued under the control of AUTOMON.

Automon

This core resident program is nearing completion and will acquire data from the animals at regular intervals of fifteen minutes. The operation of AUTOMON is shown in Figure 6. An animal can be logged on the automatic monitor by use of a special user program which can be called from the animal site. The log-on procedure includes entering the pertinent data for that animal: animal number, device number, and the programs to be used in evaluating the data. There will be a sixteen-program library available for use on AUTOMON, and any given animal can be assigned four of the sixteen programs for analysis. After the programs are selected, it will often be necessary to run a set of calibrations for the data used by those programs. The programs which are used on the automatic monitor are modifications of the user programs which have just been described. For instance, the Pressure program will be used but will have the capability of calculating the mean, maximum and minimum of at least four different pressures simultaneously. The trigger signal is sampled and by use of a special small program is made available to all programs. For example, the trigger will be used not only by the Pressure program to perform its data reduction on the four pressures, but also by the Flow program.

When the log-on procedure is complete and the information has been transferred to tables used by AUTOMON, the data from the animal will be monitored by use of an interrupt generated by the time-of-day clock. Every minute, AUTOMON receives an interrupt and makes a check to see if there is any monitoring to be done. If a new patient has just been entered, then on the next interrupt, it will be sampled and will go through a full monitor period. This includes calculation of the data, a check for alarm conditions, and storage of data. The time controlling the sampling of the animal is then updated by fifteen minutes, at which time another sample will be automatically taken.

The data reduction is carried out as shown in the bottom part of Figure 6. Any number of analog channels up to eight for any given animal are sampled at one time for a three-second period. Regardless of the number of beats during one sample period, the data is collected from each beat. The three-second sample period was selected to guarantee that at least one total heart cycle would occur during this period at heart rates down to forty beats per minute. Four different sample rates can be used for sampling the eight channels. At the end of the three-second sample period, Program 1 is called in and performs the calculations on the data associated with that program. Program 2 is then called in and performs its data reduction. This process continues for the four programs which were selected for that animal and takes approximately 2.2 seconds. The data is then sampled again for a three-second period and the programs are recalled to perform the data reduction. This process continues until six sample periods have occurred, at which time the average of all the variables calculated is taken. Then a program is loaded into memory which will check the variables for alarm conditions.

From a standardized limit table which is stored on the disk, a special limit table is generated depending on the variables selected for each animal at the time he is logged on the automatic monitor system. This table then provides the information for the alarm check. If the variables are outside the range specified and an alarm condition exists, an indicator is illuminated at the Main Monitor Console in the monitor room and the data is especially tagged for future reference.

Associated with the automatic monitor is a *"Measure Once"* option which allows any animal logged on the automatic monitor to be sampled on a demand basis. The data sampling and analysis is carried out as though it were one of the regular fifteen-minute samples. It is checked for alarm conditions and stored but does not interfere with the regular automated sample interval.

To review any of the data which has been collected by the automatic monitor, the On-Line Data Review program is used.

SUMMARY

A real-time, time-sharing computer system has been described which is currently being set up and used in the Utah Biomedical Test Laboratory in Salt Lake City. The system presently is used almost exclusively for the analysis of animal data obtained in that Facility, although it does have the capability to expand to include the analysis of engineering data or *in vitro* test data by merely adding the proper program to the user program library.

The on-line feature of this computer system allows direct entry of data into the computer without manual intervention and at the same time stores the data in a well-organized file structure so that it can be retrieved immediately for review or for further analysis later. This eliminates the need for the customary review of rolls of analog data which are stored either on analog tape recorders or on oscillographic recorders.

The computer performs calculations which otherwise would not be done or would require hours of calculation time. It provides instant feedback of information which is being obtained. This feedback helps determine the direction of the experimental procedure while the experiment is in progress and provides more efficiency in obtaining more significant data.

It has an automatic monitor feature which allows several animals to be monitored on a periodic basis twenty-four hours a day. Since the monitor is essentially under the control of one operator, the necessary personnel requirements are reduced for maintaining a high level of monitoring of animals.

In conjunction with the data system, the computer aids in detecting meaningful alarm conditions and allows analog recording to be done only at the times when it is most significant.

The use of a real-time, time-sharing computer in the animal laboratory can reduce data handling errors, increase monitoring capability and efficiency, and provide greater insight into animal tests and experiments as they are performed.

REFERENCES

1. Topham, W. S., and Warner, H. R. The Control of Cardiac Output During Exercise. Chapter 4, *Physical Basis of Circulatory Transport*, W. B. Saunders Company, 1967.
2. Warner, H. R. Control of Circulation as Studied with Analog Computer Techniques. Section Z: Circulation, Volume III, *Handbook of Physiology*, 1965.
3. Warner, H. R., and Pryor, T. A. Time Sharing in Biomedical Research. Datamation, April, 1966.
4. Pryor, T. A., Gardner, R. M., and Day, W. C. Computer System for Research and Clinical Application to Medicine. AFIPS Conference Proceedings, Vol. 33, 1968.
5. Stauffer, W. M. Computers in Physiological Analysis and Simulation. Clinical Pharmacology and Therapeutics, Vol. 8, Jan–Feb 1967.
6. Warner, H. R., et al. Quantitation of Beat-to-Beat Changes in Stroke Volume from Aortic Pulse Contours in Man. J. of Applied Phys. Vol. 5, March 1953.

DISCUSSION

J. N. Martin, "Medical Electronics News," Philadelphia: You mentioned that the terminal was exceptionally good despite its price but you didn't tell us who makes it and how many you use.

Dr. Topham: The terminal is made by Beehive Medical Electronics which is a firm in Salt Lake City and at the present time we have four terminals in the facility. We have the capability to expand that up to sixteen terminals depending on what our requirements become in the future.

D. B. Jackson, Abbott Laboratories, North Chicago: What sorts of alarm conditions did you encounter? What types of alarms?

Dr. Topham: We have two sets of alarms.

One is generated acutely, which means that if we get a cardiac arrest, the alarm is generated at the patient monitor console itself and is not under computer control. And so, if there is a life-threatening situation then this alarm would be generated. If it's a trend type problem in which we see a gradual deterioration of pressures or cardiac outputs or changes in peripheral resistance which is detected by the computer, then the computer will turn on the alarm. We have those two types of alarms to watch. One is a physiological trend and the other is an acute immediate alarm that we have to attend to and is not computer controlled.

REVIEW OF ANIMAL INSTRUMENTATION: TRANSDUCER CAPABILITY

W. S. Topham*

In almost all animal experiments designed for evaluation of the cardiovascular system, the measurement of flow, pressure, electrical activity of the heart (ECG), and blood gasses, including oxygen saturation and pH, are important. While the electrocardiogram is the most frequently measured variable, it is somewhat more difficult to obtain in animals. Experience has shown that externally applied electrodes are more reliable than needle electrodes. Accurate and calibrated *in vivo* flow has been difficult to obtain, particularly if instantaneous flow measurement is desired. However, the electromagnetic flowmeter now has pre-calibrated probes available and an accurate electrical zero which allows zero flow to be determined at any time. This adds a new dimension to *in vivo* flow measurement. To accurately measure pressure, a catheter tip transducer is required; but because of the temperature sensitivity of the transducer, it is difficult to calibrate the transducer after implantation. A method for reducing the temperature drift is described, and accurate venous pressure measurements obtained while a dog is exercising are possible, using a pulsed excitation source for the catheter tip transducer. Frequent oxygen saturation measurements are difficult and somewhat tedious to perform when blood has to be drawn for each measurement. A fiberoptics oximeter using a 5-French catheter with a center lumen can give continuous oxygen saturation measurement, as well as pressure measurement. One catheter provides hemodynamic and blood gas measurement.

INTRODUCTION

To evaluate the function of the cardiovascular system or the effects that circulatory assist devices have on this system, measurement of pressure, flow, electrical activity of the heart (ECG), blood gas content, and pH are of great importance. The particular flows and pressures measured are dependent upon the experiment being conducted or the device being evaluated. For instance, if the device under test is to increase or maintain the cardiac output and coronary perfusion while at the same time reducing the workload on the heart, then coronary blood flow, aortic pressure, and total flow in the aorta become of particular importance. The ECG is a good indicator of the rhythm and electrical timing of the heart. This information is particularly important if the heart has been insulted or if it is necessary that an assist device function synchronously with the heart beat. Blood gas measurements provide information as to whether the circulatory system is carrying out its function of oxygen transport and CO_2 removal. Oxygen saturation measurements give indication of the effectiveness of the circulatory system in performing these functions, but measurement of the partial pressure of oxygen, CO_2 and pH provide added insight. Since one or more of these measurements, flow, pressure, ECG, or blood gas, is basic to cardiovascular experimentation, it will be the purpose of this paper to describe some methods and limitations in obtaining accurate measurement of these variables.

ECG MEASUREMENT

The ECG is probably the most frequently measured parameter because it can be obtained with non-invasive techniques. However, its simplicity of measurement is reduced in animal work because of the difficulty in obtaining good electro-contact through the fur of the animal. While subcutaneous electrodes are suggested, obvious and simple surface electrode methods have provided good ECGs in our animal experiments. In exercising dogs, it was found necessary to shave the contact area very closely, either with a safety razor or a fine set of clippers, and clean the area with a surgical scrubbing brush, scrubbing not only to clean the area, but

* Manager, Dept. of Biophysics, Utah Biomedical Test Laboratory, Salt Lake City, Utah.

to flush the skin which improved the electrical signal. Standard spot electrodes were covered with electrode paste and applied to the skin with circular adhesive pads. This technique permitted us to obtain a good ECG for several hours while the dog was active and exercising on a treadmill. The slight irritation to the skin seemed to be particularly important to reduce the impedence between the animal and the electrodes. If the impedence were not reduced in this manner, then noise was introduced on the signal because of the motion of the wires as the dog exercised.

If the dog is anesthetized or otherwise immobile, a good ECG signal can be obtained by coarsely shaving the upper part of the two front legs, washing the area with alcohol, applying large amounts of electrode paste, and taping spot electrodes over the paste. The ground electrode is generally connected to the abdomen or hind leg in a similar manner. The ECG signals obtained are very adequate for triggering and rhythm determinations and have been found to be more reliable than needle electrodes.

FLOW MEASUREMENT

Measurement of *in vivo* flow has always been difficult. The two standard methods referred to in almost every physiological textbook,[1,2] the indicator dilution method and the Fick method, use equations from which a direct measurement of flow can be obtained. For the measurement of cardiac output, both methods require the insertion of at least one catheter in the venous system and a catheter or needle in the arterial system. In addition, the Fick method requires that the total amount of gas breathed by the animal be collected in order that the oxygen-consumption-per-unit time can be determined. This procedure is very difficult in an animal which is awake, but for an anesthetized animal, the endotrachial tube provides a convenient method for collection of the respiratory gas. A sample of thoroughly mixed venous blood must be taken to guarantee accuracy of the measurement, and the placement of the catheter should be in the pulmonary artery. The indicator dilution method requires thorough mixing of the dye and the blood between the point at which the dye is injected and the sampling site where the concentration of dye is measured. In the body the heart and lungs provides the most thorough and complete mixing chamber, and the dye is injected into the right heart. Its concentration is sampled at an arterial site in the systemic circulation. Since the total cardiac output passes through the injection site, direct measurement of cardiac output is obtained.

More recently, thermal dilution curves have been used to measure cardiac output and are being used more and more because they require the insertion of only one catheter, which serves as the injection site for a temperature-controlled saline solution, as well as the sampling site.[3] The saline is quickly dispersed within the body and does not offer any of the problems of recirculation or the buildup of dye concentration as is seen with the indicator dilution curve using indocyanine green dye.

All three of the above methods are an averaging process and give an average measure of flow over several seconds. They cannot measure instantaneous flow. In addition, they are most successful in measuring total cardiac output. The dilution method could be used to measure regional or organ flows, providing adequate mixing of the indicator could take place between injection and sampling sites. However, this method and the Fick principle are awkward for these special flow measurements; and it is often desirable to observe the change in flow during a heart cycle or at least obtain a mean flow measurement with each heart beat, such as, stroke volume. Even though stroke volume can be determined by the above measurements by dividing the cardiac output by heart rate, only the average stroke volume can be obtained, and transient beat-to-beat changes cannot be detected if present.

Electromagnetic Flowmeters

These devices were developed in 1936 by Kolin,[4,5] but did not become popular because of their bulky size and awkwardness in use. It was necessary to use large D-C magnets and externalize the artery to make a measurement using the electromagnetic method. By 1959, the

flowmeter had been reduced in size and could be used to measure flow in arteries as small as 1.5 mm in diameter.[6] The development of the Doppler ultrasonic flowmeter gave similar measurement capability.[7]

These two methods of measuring flow are widely accepted. They both require the placement of a flow probe around the vessel in which the flow is to be measured, and they have the capability of measuring instantaneous flow in almost any main vessel in the cardiovascular system. Comparison tests of the two methods showed that they both gave nearly identical waveforms, but each had a limitation.[8] It was very difficult to obtain a reliable electrical zero in the electromagnetic flowmeter, and the Doppler ultrasonic flowmeter did not give a reverse flow measurement. Other reports indicate solution of both of these problems. Folts demonstrated that a reliable zero for the electromagnetic probe could be obtained in animals with implantations of up to twenty-eight days.[9] Recent comparisons of a directional-sensitive Doppler ultrasonic flowmeter with an electromagnetic flowmeter have shown no differences in the waveforms,[10] although the Doppler flowmeter does tend to be more sensitive to disturbance in velocity flow profile than does the electromagnetic flowmeter. Another major difference between these two methods of measuring flow is the amount of power required to drive the probe. The Dopper flow probe uses very little power and has thus been used in extensive studies in which telemetry has been used to telemeter the information from free-roving animals to a central recording station. This is very difficult with the use of electromagnetic probes because of the power necessary to generate the magnetic field across the vessel.

The electromagnetic flow probe has become a standard method of measuring flow, and when compared to other methods in *in vitro* tests, has proven to be a very reliable and accurate method of flow measurement.[11] One of the major problems encountered when using the electromagnetic flowmeter has been that of calibration. It has been necessary to perform an elaborate external calibration procedure or calibrate internally using a direct flow measurement method such as the indicator dilution or the Fick method, and these methods may not be as accurate as previously thought. Electromagnetic flow probes are now available which have been previously calibrated and are guaranteed accurate within ± 5 per cent, provided they are applied properly to the vessel.[12] This application includes a thorough cleaning of the electrodes and, in acute preparations, a slight constriction of the vessel (10 to 20 per cent) will guarantee good electrical contact between the vessel and the electrodes. In chronic implantation, the probe should be slightly larger than the vessel, and measurements should not be made until the adventitia has grown and made good contact with the electrodes—about two weeks later. Another important improvement in electromagnetic flow measurement is the electrical or non-occlusive zero. This feature allows zero flow to be observed any time the flowmeter is being used by turning a switch on the flowmeter; thus, zero flow from chronic implants is readily available and direct flow readings can be made using the precalibrated probes once zero flow is known. Tests of the electrical zero against an occluded zero are found to be very accurate. The results of these tests using several different flow measurements are shown in Figure 1. In all cases, the electrical zero was set prior to the occluded zero being measured. These two features, electrical zero and pre-calibrated probes, have improved the flow measurement capability of the electromagnetic flowmeter significantly and have made it a valuable direct flow-reading method.

There are many commercial flowmeters available; however, the cost ranges from $2,000 to $3,000 per channel, and before purchase is made, a careful evaluation of the instrument should be made. While *in vitro* tests are good to measure the linearity and some flow characteristics of the flowmeter, *in vivo* tests should be conducted for a complete evaluation. Such items should be considered as the stability of the baseline, the reproducibility of the flow measurement by removing the probe from the vessel and replacing it, the sensitivity of the flow probe to orientation on the vessel, and the amount of other electrical noise, such as ECG, which is picked up by the flow probe. When the tests are being conducted, the flow probe should

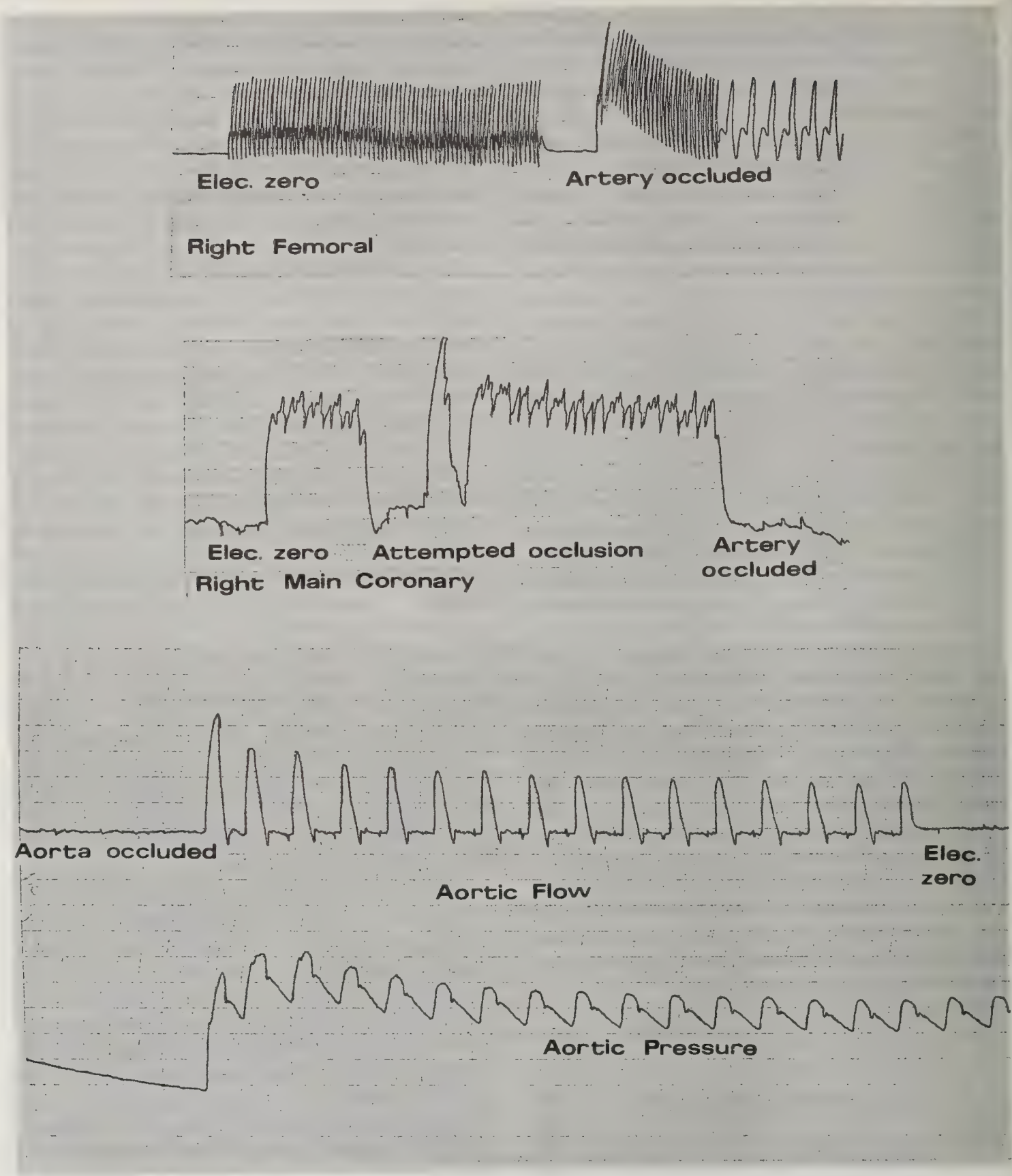


FIGURE 1.—Flow measurements showing the electrical or non-occlusive zero compared to the occlusive zero in the femoral and right main coronary artery and the aorta of a dog.

fit snugly on the vessel, and care should be taken to make sure that there is good electrical contact between the electrodes and the vessel wall. Under these considerations, three commercially available flowmeters were evaluated by us. A sample of resulting waveforms obtained from the ascending aorta by these flow probes is shown in Figure 2. Trace 'a' shows an ECG breakthrough, and there is significant signal due to the ECG with each heart beat. Trace 'b', taken from a different manufacturer's flowmeter, shows a highly unstable baseline, and zero flow is not evident during diastole. Trace 'c' is an accurate measurement of ascending aortic flow. The baseline is stable, zero flow is measured during diastole, there is no ECG noise, and the waveform appears correct. All of these tracings were taken in acute preparations and are representative of three experiments. All flowmeters were used during each experiment.

PRESSURE MEASUREMENT

In vivo pressure measurement is most often done by inserting a catheter into the bloodstream, and then connecting the end of the catheter to a transducer which is mounted near the animal. The transducer is sometimes taped to the side of the animal, particularly if he is to move around or exercise on a treadmill. This method of pressure measurement has some inherent problems, such as the perturbations introduced on the pressure waveform due to the movement of the saline column within the catheter. The frequency response of the system is also reduced because of the added mass of the fluid in the catheter. The reduction in frequency response is generally not a serious problem, although it has been pointed out that if the rate of change of pressure with respect to time (dP/dT) is to be evaluated, the frequency response of the system must be carefully considered.[13] It has been reported that the frequency response of the system can be increased by flushing the catheter with CO_2 immediately prior to its being filled with saline before use in the pressure measurement system.[14]

The ideal way to measure pressure would be to measure it at the point at which it occurs without an intervening transmission system such as the saline column in the catheter. This can be done with catheter tip transducers. These transducers have been available for many years, but have not gained wide acceptance for *in vivo* testing because of their inherent problem of temperature drift and because there is no way to check the zero of the transducer after it has once been implanted. This problem has been solved by some pressure transducer manufacturers by providing a lumen through the catheter on which the catheter tip transducer has been fixed. Thus, with a single catheter in the system, the transducer can be calibrated externally, and then the measurements during the experiment can be obtained from the catheter tip transducer. If the transducer could be calibrated at the time of the implantation, this procedure would not be necessary. However, if the medium surrounding the transducer changes temperature, a drift will occur. This drift is not as significant as that caused by the internal heating of the transducer from the excitation voltage. It can be shown that when the velocity of the flow increases across the transducers sensing diaphragm, then the heat that has been generated is dissipated at a higher rate.[15] This, in effect, reduces the temperature of the gauge and causes a false deflection, indicating a reduced pressure. This makes the pressure transducer sensitive to flow variations, and if the flow is to change during an experiment, it can be a serious problem.

Venous Pressure

One of the most difficult pressures to measure is venous pressure while the animal is exercising on a treadmill. This pressure is so low it cannot be measured by using an external pressure transducer and catheter system because the motion artifact is so great as to completely destroy the accuracy of the venous pressure measurement. A catheter tip transducer must therefore be used, but the changes due to flow perturbations and temperature changes are again as large as any changes which would be observed in the venous pressure. In order to guarantee that meaningful pressure data is being obtained, these artifacts should be re-

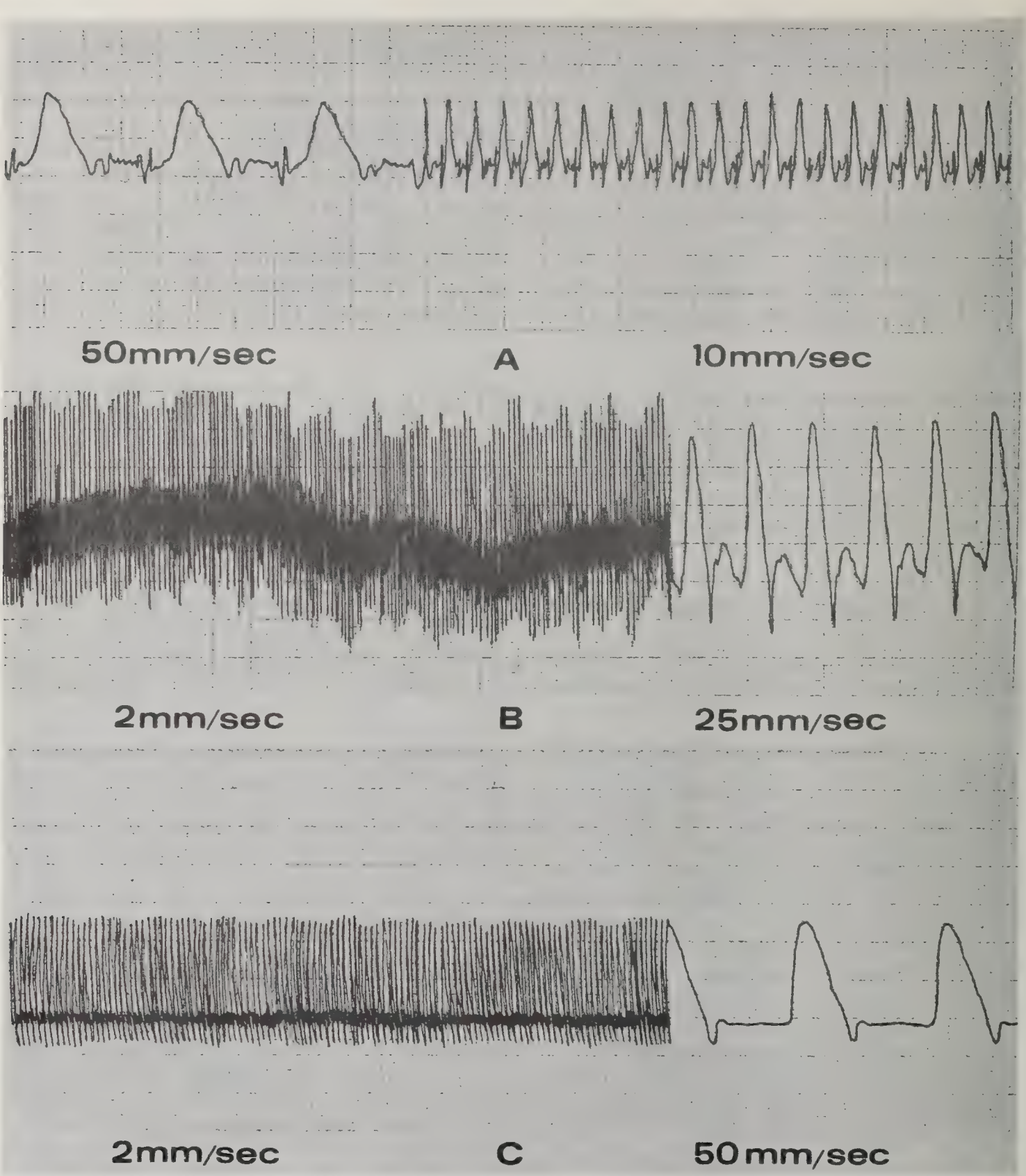


FIGURE 2.—Typical waveforms of three different manufacturer's flowmeters. Probes' fit was good in all cases and three experiments were performed with all flowmeters used each time. (A) Note strong ECG signal. (B) The baseline is unstable and zero flow during diastole is not evident. (C) Accurate measurement or aortic flow.

duced to an absolute level of ½ cm/H_2O. To achieve this accuracy, a new method was developed in our laboratory, and the results have been reported.[16,17]

A Statham SF-1 transducer was selected because it had a lumen in the catheter for external pressure measurement. This transducer was selected even though it was more sensitive to temperature than other transducers which are currently available because *in vivo* calibration was essential without the insertion of other catheters.

A plot of temperature versus voltage output was taken at a zero pressure, and it was found that there was a linear relationship. The sensitivity was 7 cm/H_2O per degree Centigrade change. Since it was assumed that the temperature in the exercising dog would be expected to vary at least one degree, this variation had to be compensated. This was done by attaching a thermistor to the tip of the catheter, as is shown in Figure 3. With the thermistor giving the actual temperature at the catheter tip, the correction could then be made in any drift due to blood temperature change at the time the data was analyzed.

To check for the second source of zero instability, due to internal heating, the catheter was submerged in a saline bath and the saline was stirred to provide a flow motion past the tip of the catheter. With the temperature maintained at a constant value, the stirrer was turned on and off, and it was found that there was a shift in the pressure reading of 5 to 10 cm/H_2O. This sensitivity of flow was expected because at the suggested power input, the metal tip of the catheter would get warm to the touch if left in air. The obvious way to eliminate this problem is to reduce the amount of power input to the transducer; however, reductions of the excitation voltage by a factor of five or ten, which would be necessary to effectively eliminate the drift, would provide a pressure transducer sensitivity of 1 to 2 microvolts per cm/H_2O. This low voltage output would negate any increase in accuracy because of the noise problem in the amplifier. Another way to reduce the power input is to apply the same voltage level, but re-

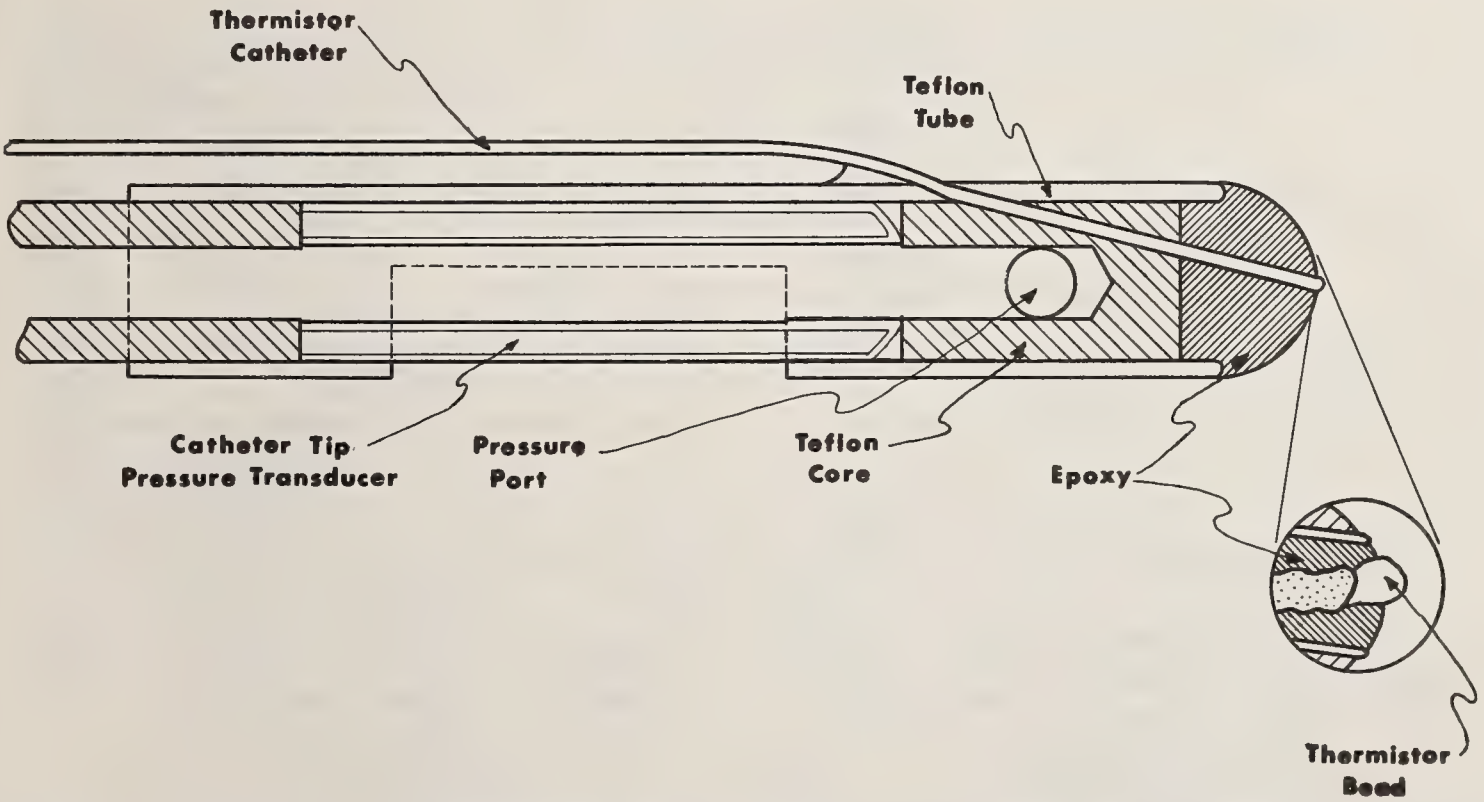


FIGURE 3.—A Statham SF-1 catheter with a thermistor attached for temperature measurement of surrounding fluid. The thermistor provides a signal for correction in temperature drift of zero pressure.

duce the time when the voltage is applied. This can be done by applying voltage pulses of short duration compared to the period between them. The power input will then be greatly reduced. Using this method, the transducer output can be sampled only during the excitation period of the pulses, and the pulse rate will determine the frequency response of the system. Because the highest frequency in physiological pressure signals is 25 hz, a pulse rate of 200 pulses/sec was decided upon. The pulses had a duration of 200 (microsec.) and were 5 volts in amplitude.

The average power input to the transducer was reduced by a factor of 25 below that of a 5-volt D-C excitation. A diagram of the system used is shown in Figure 4. From the pulse generator, the pulse is fed into a pulse filter and amplifier. The amplifier is used to provide sufficient power to drive the transducer bridge, and the pulse filter was found necessary in order to round off the sharp edges of the pulse by filtering the pulse at frequencies lower than 10 kilocycles. This was necessary because slight differences in pulse shape detected by the differential amplifier from each side of the transducer bridge could drive the amplifier into saturation. The pulse was also delayed and narrowed and used as the trigger for the track and hold circuit which samples the output voltage during the excitation period. This method also allowed

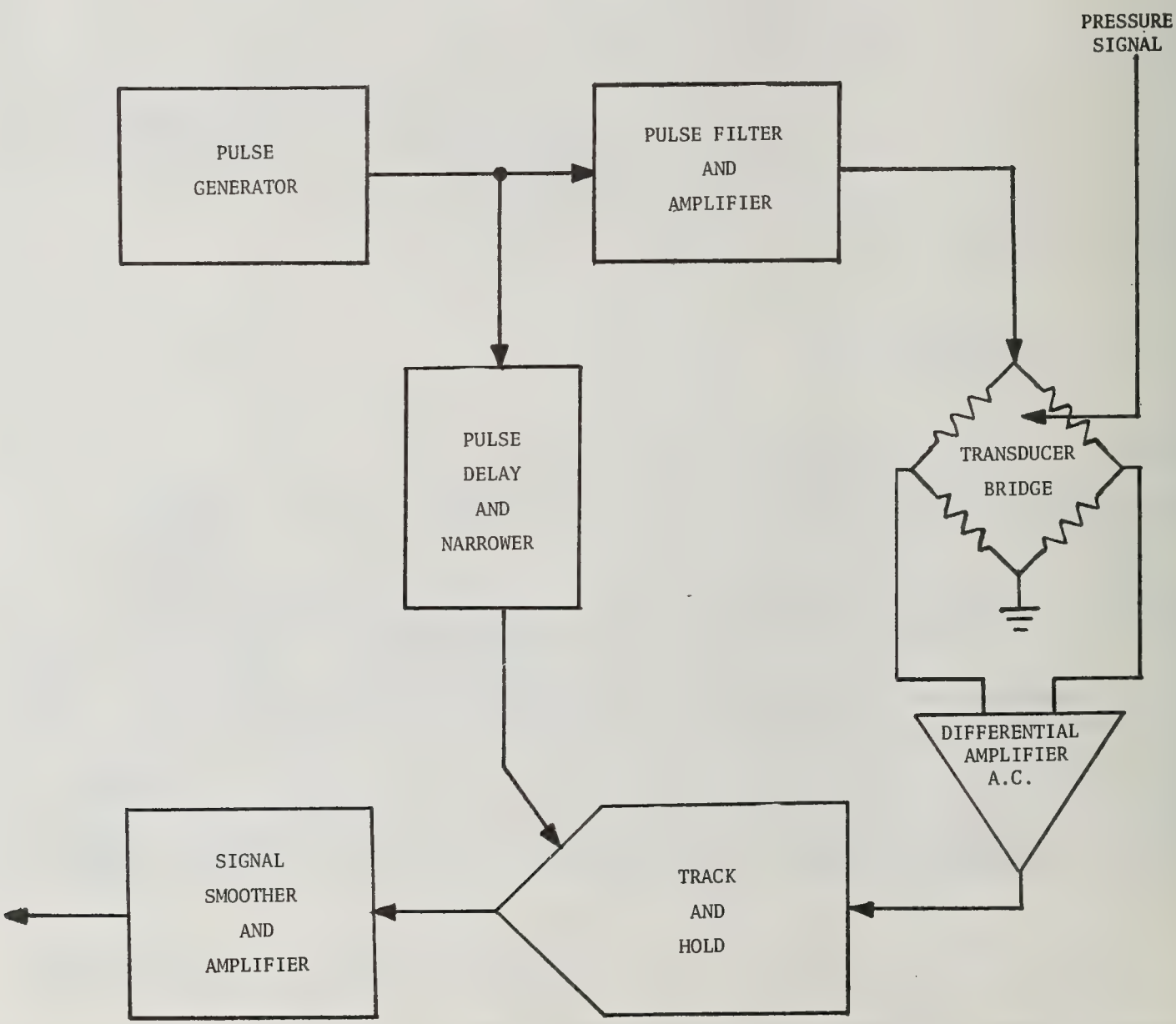


FIGURE 4.—Functional block diagram for a pulse-driven catheter tip pressure transducer.

the use of an A-C amplifier rather than using a D-C amplifier, which has its own inherent problems of drift and zero stability. The resultant pressure signal was indistinguishable from that of the D-C excited system.

To test the system, the gain of the amplifier was adjusted to produce a 0.2-volt output per cm/H_2O, and the transducer was immersed in a saline bath, which was kept at a constant temperature. A magnetic stirrer was then turned on and off to check the transducer sensitivity to flow dependent heat transfer. The transducer was first checked using the standard D-C excitation system, and the results are shown in Figure 5, Part A. The same transducer, under the same conditions, with only a very slight change in the bath temperature, was again tested. It can be seen in Figure 5B that the zero drift was reduced to just over 0.5 cm/H_2O, as compared to 7 cm/H_2O under the D-C excitation conditions. This technique seems to solve the zero drift problem due to flow perturbations, but it was found that because of the dependence of the pulse on the capacity characteristics of the transducer, the transducer became more sensitive to the type of fluid inside the catheter because it affects this capacitance. Thus, when calibrating the transducer, it is necessary to use the same fluid as is used during the experiment. Based on these *in vitro* tests and making a correction for any temperature change of the environment around the transducer, it was determined that this transducer had an accuracy of 0.5 to 1 cm/H_2O.

The purpose in developing the catheter was to facilitate accurate measurement of venous pressure and to record the relatively small changes in this pressure as a dog exercised on a treadmill. Under local anesthetic and with the dog fully awake and standing on the treadmill, two catheters were inserted into the right jugular vein. One catheter was advanced into the right atrium, and the other advanced on into the inferior vena cava as was verified by fluoroscopy. Typical waveforms obtained during an exercise run are shown in Figure 6. A typical right atrial pressure waveform is shown prior to exercise. The pulsations are greatly reduced at the inferior vena cava, and while there may be some artifact introduced on the pressure

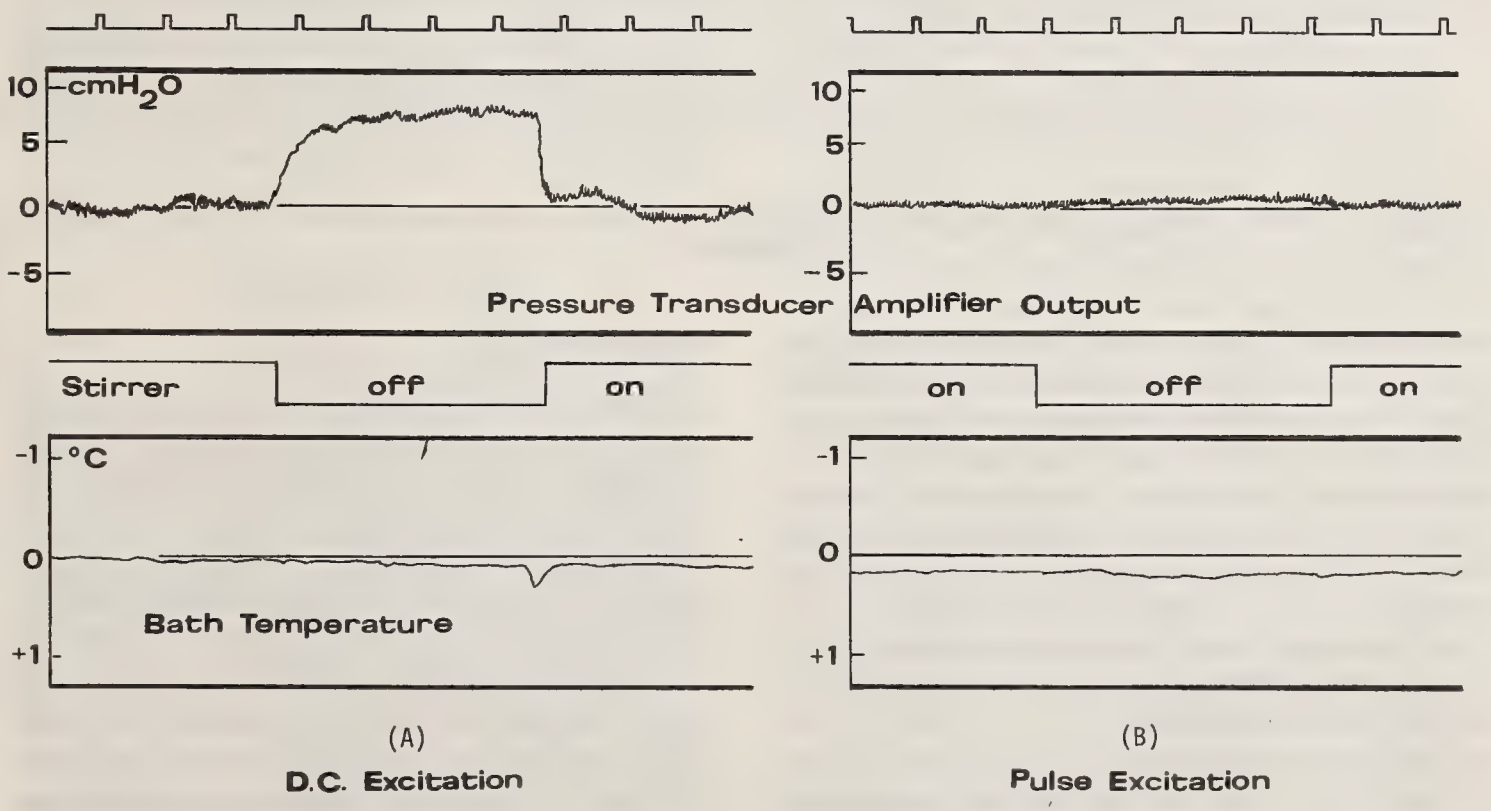


FIGURE 5.—Results of an *in vitro* test showing the zero drift due to temperature variation caused by flow changes. The standard D-C excitation and the new pulse excitation method are compared.

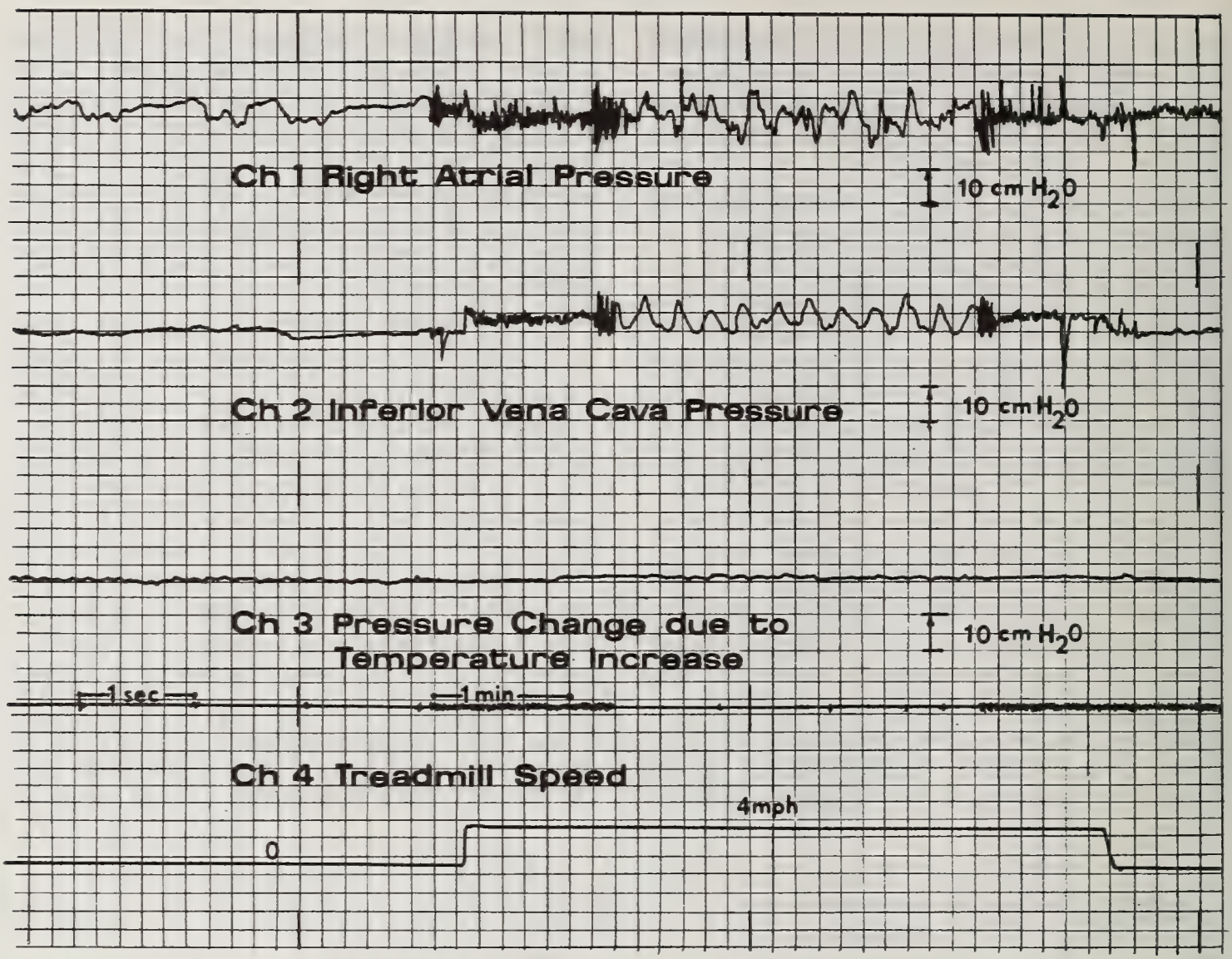


FIGURE 6.—Venous pressure changes in an exercising animal as measured with pulse-driven catheter tip pressure transducers.

during exercise, the waveforms look clean, and we can be assured that the mean level shifts that we see are due to actual pressure changes and not due to the change in zero because of temperature instability. The change in pressure due to blood temperature change is very small, as is shown in the Channel 3 tracing. The mean increase of inferior vena cava pressure at the onset of exercise and the mean decrease of right atrial pressure was typical of the responses observed in this experiment.

This new method of pulsing catheter tip transducers can be used for any such transducer and would provide zero stability for *in vivo* applications.

Arterial Pressure

A single catheter inserted into the aortic arch can provide much information about the cardiovascular system. Using the method of Warner,[18] stroke volume can be calculated from this aortic pressure waveform, and in turn, heart rate, cardiac output, mean pressure, and peripheral resistance can also be observed on a beat-to-beat basis. In addition, it is possible to determine systolic duration, stroke work, and obtain a measure of dP/dT. While thi· information can be obtained by the insertion of only one catheter, it does require sophisticated analysis of the pressure waveform which at the present

time is done on a general-purpose digital computer.

During recent experiments,[16,19] it was desired to have the animal in as normal state as possible while data was being obtained. Therefore, a technique was developed which allowed the insertion of an arterial catheter without anesthetizing the animal. This technique involves injecting a local anesthetic into the neck of the dog. After the anesthetic has taken effect, a small mid-line incision is made and the right or left carotid artery is disected out. Care must be taken not to stimulate the vagus nerve as the carotid sheath is cut and the artery and nerve are separated. In a dog, the distal portion of the artery can then be tied off without any ill effects. A small incision is made in the artery and a catheter is inserted until a good central aortic pressure waveform is obtained. The insision is then sutured closed and the catheter tied. The animal can be used immediately for experimentation such as exercise runs on the treadmill. Since the catheter is tied in by the suture and is flushed daily with heparinized saline, the preparation will last for several days, and repeated experiments can be performed. This technique was also used for the insertion of catheter tip transducers, thus allowing more accurate measurement of the central aortic pressure and eliminating any distortion which might be caused by catheter movement in relation to an external pressure transducer.

OXYGEN SATURATION

The partial pressures of blood gases can be measured using standard clinical laboratory instruments, as can pH. The per cent of hemoglobin saturated with oxygen (O_2 saturation) can also be measured in the laboratory, but more rapid methods for on-line use have been developed. One of these methods requires the use of a cuvette oximeter, through which a sample of blood is drawn. The intensities of transmitted IR (805 mμ) and R (630 mμ) light is measured from the output of selenium oxide photoelectric cells. The ratio of these intensities is logarithmically related to O_2 saturation, and by using a two-point calibration, the saturation can be obtained. The logarithmic equation can be solved by using analog circuitry and a meter readout, or voltages proportional to the light intensity can be fed directly into a computer for on-line calculation and data storage. This latter method has helped in reducing the manual handling of data and provided an instantaneous digital readout.

Cuvette oximetry has been used for many years, and while it provides a rapid method of determining O_2 saturation, a blood sample must be drawn through the instrument and continuous measurement is very difficult. In many circumstances, continuous measurement without withdrawing blood samples is desirable either because the rapid time course of saturation is needed or because the loss of blood is detrimental to the experiment. This problem has been solved using fiberoptics and the reflection of light from the bloodstream.[20,21,22] With the use of light-emitting diodes and solid-state photosensitive devices, the method has been improved, and recently a solid-state fiberoptic oximeter was reported.[23,24] The oximeter uses a 5-French catheter with a center lumen and the fiberoptics positioned around the lumen. It is this catheter that makes the unit particularly useful in experiments. With the insertion of one catheter in the aortic arch, not only can continuous O_2 saturation be measured, but blood samples can be drawn for blood gas and blood chemistry determination. In addition, central aortic pressure can be measured and hemodynamic variables can be calculated as stated previously. With an additional catheter in the central venous system, venous saturation can be continuously monitored and an A-V difference calculated. From this difference and an O_2 consumption measurement, cardiac output can be calculated using the method of Fick. Also, the central venous pressure can be measured. It is possible to calculate cardiac output using the indicator dilution method if dye is injected into the venous catheter and the arterial catheter is used to detect dye concentration. This would require a change in wave length of the IR light-emitting diode from 900 mμ to 805 mμ, and although such a diode is available, it has not been used in the present system.

If this method of O_2 saturation measurement is used in animal experiments, not only are the

advantages of continuous measurement available, but with two catheters in place, several additional hemodynamic variables can be monitored, including cardiac output, arterial pressure, and central venous pressure.

SUMMARY

Measurement of basic cardiovascular parameters, pressure, flow, ECG, and blood gas (O_2 saturation and pH) is discussed. A simple but reliable method for obtaining the ECG is described. A new and more accurate method of measuring venous pressure under exercise conditions by using a pulsed catheter tip transducer is available. In flow measurement, there are average direct measurement methods of determining *in vivo* flow, particularly cardiac output, but instantaneous flow measurement methods (electromagnetic flowmeter, Doppler ultrasonic flowmeter) have become practical, reliable methods with recent improvements. The electromagnetic flowmeter now has two features, precalibrated probes and an electrical or non-occlusive zero, which give it the ability to directly measure any *in vivo* flow.

A new solid-state fiberoptics oximeter has not only the advantages of continuous O_2 saturation measurement without withdrawing blood samples, but also the capability for measurement of pressure through a lumen in the catheter.

REFERENCES

1. GUYTON, A. C. *Textbook of Medical Physiology*, 4th Ed., W. B. Saunders Company, 1971.
2. SELKURT, E. E. *Physiology*, 2nd Ed., Little, Brown and Company, 1966.
3. SWAN, H. J. C. Hemodynamic Measurements. Talk given at Laboratory Procedures and Decision-Making in Cardiology: A Symposium, L. D. S. Hospital, Salt Lake City, Utah, March 1971.
4. KOLIN, A. Electromagnetic flowmeter: principle of method of its application to blood flow measurement. Proc. Soc. Exper. Biol. Med. 35:53, 1936.
5. KOLIN, A. Electromagnetic recording flowmeter. Am. J. Physiol. 119:355–356, 1937.
6. KOLIN, A., and KADO, R. T. Miniaturization of the electromagnetic blood flowmeter and its use for recording of circulatory responses of conscious animals to sensory stimuli. Proc. Nat'l Acad. Science 45:1312–1321, 1959.
7. FRANKLIN, D. L., et al. Technique for radio telemetry of blood flow from unrestrained animals. Am. J. Med. Electronics 5:24–28, 1966.
8. VATNER, S. F. Simultaneous comparison and calibration of Doppler and electromagnetic flowmeters. J. Appl. Physiol. 29:907–10, December 1970.
9. FOLTS, J. D. Electronic zero for chronic application of electromagnetic flowmeter probes. J. Appl. Physiol. 28:237–41, February 1970.
10. LEWIS, J. D., et al. A comparative study of electromagnetic and ultrasonic flow measurements. Br. J. Surg. 58:306–7, April 1971.
11. JACOBS, R. R., et al. Determination of the accuracies of the dye-dilution and electromagnet flowmeter methods of measuring blood flow. J. Thorac. Cardiovascular Surg. 58:601–8, October 1969.
12. GORDON, A. S. *Practical Aspects of Blood Flow Measurement.* Published by Statham Instruments, 1971.
13. GLEASON, W. L. and BRAUNWALD, E. Studies on the first derivative of the ventricular pressure pulse in man. J. Clin. Invest. 41:80–91, 1962.
14. DEAR, H. D. and SPEAR, A. F. Accurate method for measuring dP/dT with cardiac catheters and external transducers. J. Appl. Physiol. 30:897–899, June 1971.
15. KONIGSBERG, E. Flow-perturbation characteristics of implantable pressure transducers. Proc. 21st A.C.E.M.B., November 1968.
16. SCHOENBERG, A. A. Venous pressure changes: their measurement and significance in exercising dogs. Ph.D. Thesis, University of Utah, 1972.
17. SCHOENBERG, A. A., and TOPHAM, W. S. Venous pressure response in exercising dogs using a pulse driven catheter tip transducer. Proc. 24th A.C.E.M.B. 13:122, 1971.
18. WARNER, H. R., et al. Quantitation of beat-to-beat changes in stroke volume from aortic pulse contours in man. J. Appl. Physiol. 5, March 1953.
19. TOPHAM, W. S. Comparison methods for calculation of left ventricular stroke work. J. Appl. Physiol. 27:767–769, 1967.
20. GAMBLE, W. J., et al. The use of fiberoptics in clinical cardiac catheterization. Circulation 31, 1965.
21. HARRISON, D. C., et al. Fiberoptics for continuous *in vivo* monitoring of oxygen saturation. Amer. Heart Jour. 71:766–774, 1966.
22. EMSON, Y., et al. *In vivo* studies with an intravascular and introcardiac reflection oximeter. J. Appl. Physiol. 17:552–558, 1962.
23. JOHNSON, C. C., et al. A solid-state fiberoptics oximeter. J. Assoc. Adv. Med. Inst. 5:77–83, 1971.
24. JOHNSON, C. C., et al. Present and future clinical applications of the fiberoptics catheter oximeter. Proc. 24th A.C.E.M.B. 13:251, 1971.

DISCUSSION

K. SAGAWA, Johns Hopkins Medical School, Baltimore: I'm very glad to hear about your ef-

fort in making very precise venous pressure measurement, and I think the same thing applies to ventricular pressure measurement during diastole. I think Konigsberg will bring up later what we and many others find: that interventricular pressure during artery diastole is quite often negative, even if the chest is open. I have seen several records, even during this meeting, showing this negative pressure. It may be a physiological problem, but to really discuss this kind of dynamics, you have to have very precise measurements. Just a comment on your effort. Thank you.

N. P. CHING, St. Vincent's Hospital, New York City, N.Y.: Two questions. One, you didn't identify the product, by manufacturer. Is there a reason? And secondly, have you had any experience with the new intervascular flowmeter that they've put on the catheter?

DR. TOPHAM: I have had no experience with the catheter tip flowmeters. There are several kinds available. Some use hot wire anemometry; others use the electromagnetic flow principle. Biotronics has made one of the latter, which was reported in the *Journal of Applied Physiology*. The only knowledge I have of those is from reading the literature.

I can give you the names of the commercial flowmeter manufacturers that we evaluated. They may want to talk to me after I get through, but the first one that I showed was Biotronics, the second one was Carolina Medical Instruments, and the third one was Statham.

P. SOMANI, Abbot Laboratories, North Chicago: We have had some experience with this new type of flow probe, and we have been very successful in recording the flow. We have used the Carolina system, too, and compared the intervascular flow measurement from extra-corporeal flow measurements and they match very well. I'd like to note that we have been using Carolina flowmeters, and we have not seen the drift that you have. We find it very stable in our hands, at least.

DR. TOPHAM: I appreciate that comment. I was very surprised myself as we evaluated these flowmeters to see that type of response. We talked with the Carolina people and asked them why it might be. In fact, I sent a copy of our records to them and asked them to give me some suggestions. Nothing seemed to come back, and I was unable to correct the problem in my own laboratory. So I could do nothing except report what I found.

E. KONIGSBERG, Konigsberg Instruments, California: I think your reduction of 25 times the power on the catheter tip flowmeter is significant in another area, namely on the sample and hold circuit, and that is with telemetry systems which are completely sealed. This can mean an appreciable reduction in power. Right now pressure transducers burn a lot of power as compared with the more passive types of instruments. So I think there would be a significance there, too.

DR. TOPHAM: Thank you. I felt that perhaps this might be a good way to excite those transducers and maybe give us a better zero as well as reducing the power into the transducer.

MR. CLARK, "Medical Electronics News," Philadelphia, Pa.: Who makes the fiber optic oximeter?

DR. TOPHAM: It is commercially available. It was developed by Dr. Curtis Johnson at the University of Washington and there are two firms which are making it, but I can't give you their names, I'm sorry.

DR. KONIGSBERG: Physiocontrols?

DR. TOPHAM: Physiocontrols is one, Dr. Konigsberg says, and I'm not sure of the other one. I know that there is some effort to sell that particular fiber optics catheter and I'm sure that we'll hear more about it, but my communication is directly with Dr. Johnson. Thank you.

A MULTI-STATION COMPUTER SYSTEM FOR ACQUIRING AND PROCESSING ANIMAL DATA

Eugene F. Uretz, John A. McClary and Steve Morgan*

This paper describes a system used to acquire both laboratory and physiological data under control of users at terminals distributed throughout the T & E Facility of the Medical Devices Applications Program of the National Heart and Lung Institute. The facility is operated by the IIT Research Institute in Chicago. The structure of the system allows data from many sources to be acquired simultaneously with complete integrity at rates up to 500 samples per second and associates contextual description with all acquired data. These descriptions uniquely identify a given data series by describing the test under which it is acquired; the animal, device and/or sample associated with the data; and the time of execution of the test. The features of the system which allow acquisition and processing to proceed simultaneously without delay to the terminal user are described. One feature is a multi-level priority structure, which allows high priority tasks such as data sampling to take precedence over lower priority tasks such as file searching. Another is a unique contextual dayfile structure which allows all data from all terminals to be mixed together in a single file while preserving the contextual identifiers associated with the data. The system also allows automatic storage of high rate physiological data on the computer disk at high priority, where it can be analyzed and processed at low priority without compromising the integrity of the system. Another feature is the assignment of priorities to users' jobs so that short computational jobs are not delayed by slower jobs. The data acquired or derived by the system is transferred once a day to a large batch processing system via dataphone where it is stored in organized files. Many months of data are held in these files where they are used for further numerical and statistical analysis, as well as for report generation. Several examples of specific applications of the system are described. These include manual logging of biochemical data, automatic read in and processing of spectrophotometer, densitometer, and coulter counter data, and Fourier analysis of blood flow and pressure.

INTRODUCTION

The system described is part of the capability developed to meet the computational needs of IITRI operated T & E Facility of the Medical Devices Applications Program (MDAP) of the National Heart and Lung Institute (NHLI). The T & E Facility's job is the test and evaluation of medical devices. The devices are varied and the tests quite comprehensive including *in-vivo* and *in-vitro* tests which may be medical, biological, mechanical, electronic, etc. Specific tests vary markedly according to the nature of the device being evaluated. Furthermore, a single experiment may involve the collection of a great deal of data on many animals. Thus, evaluation of the Dow oxygenator involved running total or partial bypasses on many animals, obtaining many biochemical data values on each animal over a period of days, and statistically evaluating and correlating all data from all animals.

The implication in developing the T & E Facility Computational capability was clear: To be effective, the system developed had to be able to reliably acquire, store and manipulate large amounts of data of a diverse nature. To keep the reliability high, it was important to aid the lab worker in the acquisition and analysis of data of all types.

The overall system developed to satisfy these computational capabilities is a two-tiered system (Figure 1). The first tier is a small real time system used to aid laboratory and scientific workers in performing experiments, in acquiring and analyzing data, and in placing suitable identifiers with all data. It can store up to one full day's data with their identifiers.

Another very important function performed by the first tier system is the periodic transmission of all data and their identifiers to the remote second tier system. The collected data can thus be entered into the appropriate second tier

* IIT Research Institute, Test and Evaluation Facility (sponsored by MDAP, NHLI) Chicago, Illinois.

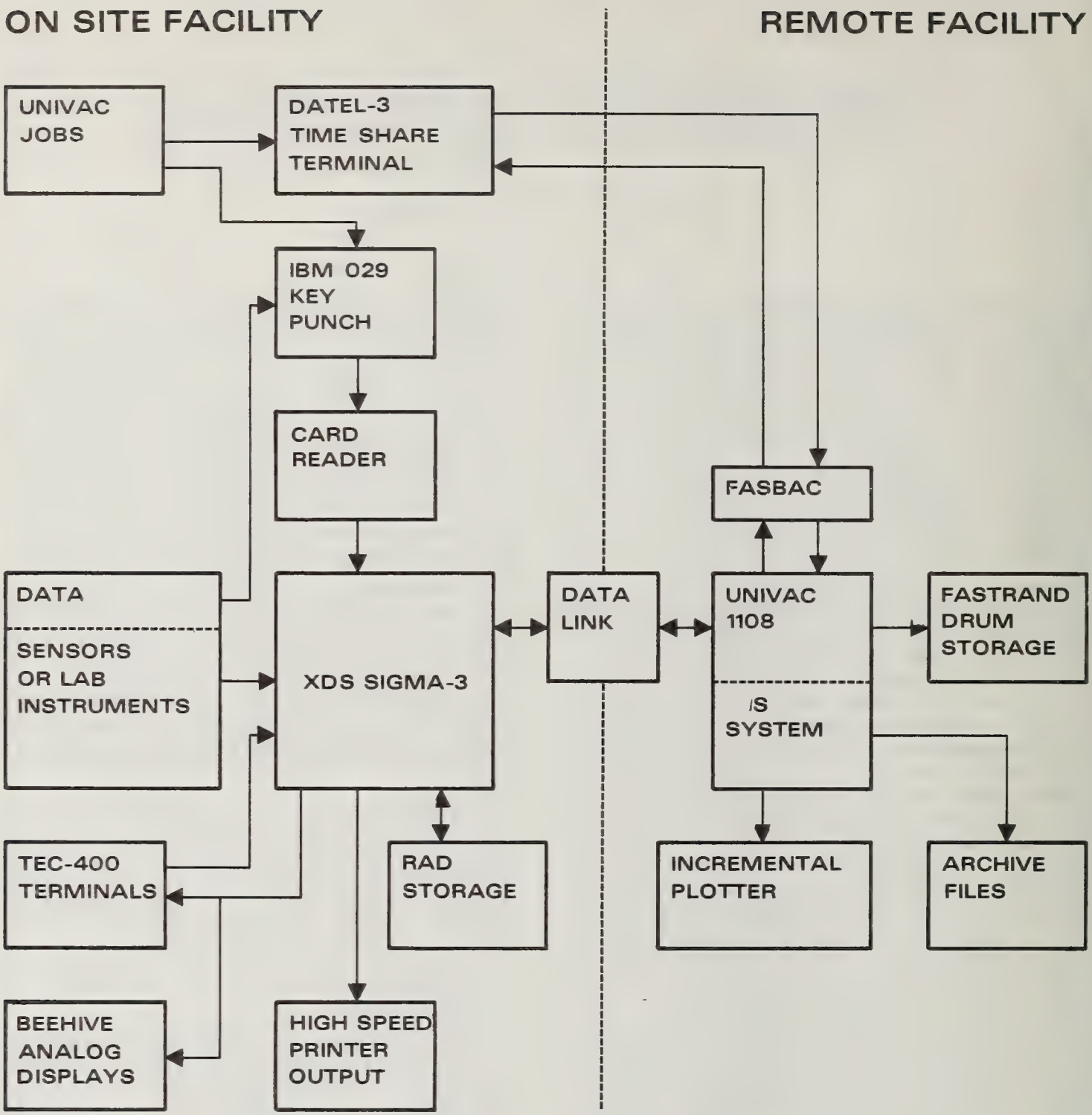


FIGURE 1.—IITRI T & E computational facility.

files without any tedious and error producing data transcriptions.

The remotely located, second-tier system is implemented on a large batch processing system (currently this is University Computing's UNIVAC 1108 system). The system software itself was developed by T & E personnel and is called the Facility Information System (FIS). It includes a large data base able to hold many months of T & E facility data in accessible form and a data base management language for acquiring, processing, manipulating and reporting this data.

We will concentrate in this paper on describing the first-tier system, the system used to aid T & E Facility personnel in performing their

experiments, acquiring data, and entering this data into the, computationally, more powerful FIS system.

Facility personnel "converse" with the Sigma-3 system by means of TEC alphanumeric terminals distributed throughout the facility. Using a simple language developed for the purpose, they are able to acquire data from lab instruments and sensors. They may also enter data values from the terminal itself. Acquired data or computed results may be displayed on the TEC terminals themselves or on Beehive Alpha-21 graphic displays. Data is stored on the system disc for later transmittal to the second tier.

The system is designed to allow all terminals to be operated simultaneously with response times commensurate with the complexity of the job being performed.

The system also acts as a high speed terminal for remote entry of batch jobs into the remote system. Cards are read and transmitted via data set to the remote system and job output is transmitted to the Sigma-3 via the data set and printed on the Sigma-3 line printer. Data can also be transmitted to the remote facility by the keypunch, card reading route. This method of data acquisition is used on tests not yet implemented on the terminal system and as a back-up method for all data entry.

SIGMA-3 HARDWARE

The facility's on-line computational equipment consists of a Xerox Sigma-3 and a number of associated hardware elements (Figure 2). The Sigma-3 has 16,000 16-bit words of random access memory and twenty distinct hardware interrupt levels. Input-output is through eight direct memory access channels or twenty-eight 16-bit digital input-output channels.

Attached to the direct memory access chan-

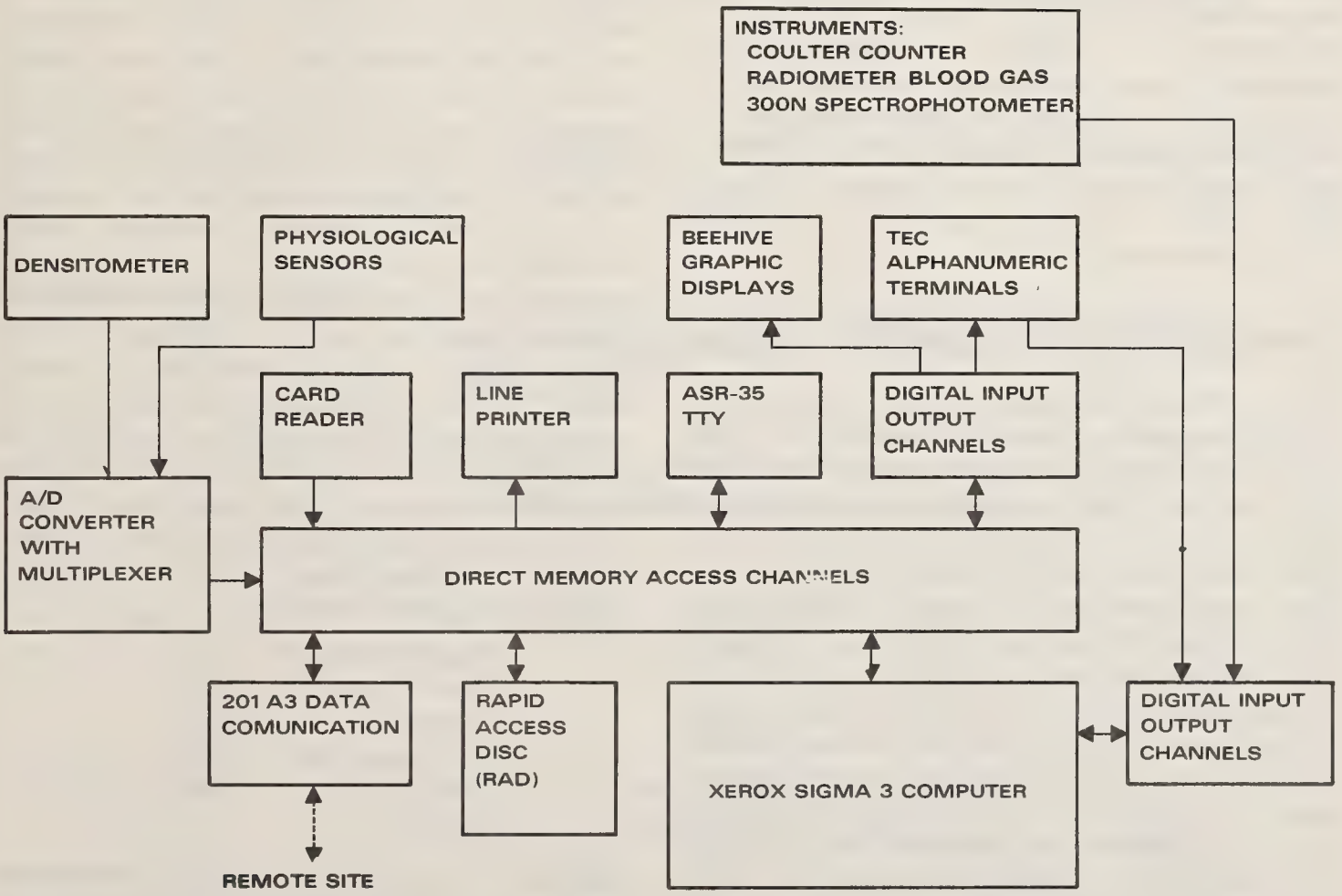


FIGURE 2.—On-site facility computer hardware.

nels are several standard peripherals: a 200 card per minute card reader, a 200 line per minute line printer, and an ASR-35 teletypewriter. Six TEC alphanumeric terminals are positioned throughout the facility. These serve as the major communication media between T & E scientific personnel and the computer. Input from the TEC's is through the digital input-output channels, since the integrated circuit memories of the TEC are used to store operator messages and these messages are read and interpreted one character at a time. The TEC memories thus act as additional computer memory.

Displays to the TEC are written at high speed through a direct memory access channel as are analog displays to the Beehive graphic terminals. The A/D converter has a maximum sample rate of 100,000 samples per second and sixty-four channels of multiplexing. It has a fourteen bit resolution with an input range of minus ten to ten volts. A Beckman Microzone Densitometer and twelve physiological channels are currently attached to the A/D converter. The system disc (RAD) has 750,000 16-bit words with an average access time of seventeen milliseconds. Several digital instruments are interfaced through the digital input-output channels. These include a Coulter Counter, Radiometer, Blood Gas Co-oximeter, and 300N Spectrophotometer.

SIGMA-3 MULTI-STATION COMPUTER SYSTEM

T & E Facility personnel control data acquisition, identification, processing and storage with TEC terminals distributed throughout the facility. The terminal language was deliberately made simple, and messages printed on the terminal help guide facility personnel in its use. This allows personnel to learn to use the computer system quickly and easily, and, thereby, allows them to concentrate on the experimental or laboratory aspects of their job. Three types of entries may be made at the terminal. These types are the context codes referred to earlier, data and special symbols. Context codes are used to describe to the computer the job to be done as well as to identify the data which will be collected. Eight context code types are available. These are shown in Table I. Keyed in data can be alphanumeric or real. The special symbols will be described as we go through an example.

System Storage Structure

Before describing the terminal language and how it controls the computer system's operation, it is necessary to describe some of the major storage structures of the system.

DAYFILE. The DAYFILE is the major data storage media of the Sigma-3. It stores up to one full day's data generated from all sources under control of the TEC terminal. At the end of a day all DAYFILE data are sent to the remote FIS system where they are entered into long term data files. Since all data are entered into the single DAYFILE structure, it is of course essential to adequately identify all data. This is done by means of context codes. Seven context code types are stored in the DAYFILE. The stored context codes have a one-to-one correspondence with the keyed in context codes but are altered in format to be more compatible with machine storage and to allow easy access to other system structures. Stored data may be fixed point, floating point or alphanumeric. All DAYFILE entries include an additional identifier which specifies the number of the terminal which caused the generation of the entry. Table II is a stylized illustration of a typical dayfile sequence. For compactness, this illustration assumes entries from only two keyboards. Each data item from a given terminal has its context defined by the first previous context of each type filed in front of it. Initially all contexts for all terminals are set to zero. If a given item of data is identified by a zero context type, it simply means that the context type is not applicable to the data item. Thus, in Table II, the data value 7.437 has the contexts:

Specimen Date	1115
Event Time	1102
Animal #	123
Specimen Time	1005
Test #	43401
Staff #	22

There is no device number assigned to this data item since the device code has a 0 value.

REFERENCE FILE. The REFERENCE

TABLE I.—*Context Codes*

TABLE 1 - CONTEXT CODES

CODE TYPE	CODE FORMAT
ANIMAL CODE	A FOLLOWED BY A NUMBER FROM 0 TO 4095
TEST CODE	T FOLLOWED BY UP TO FIVE DECIMAL DIGITS
STAFF CODE	S FOLLOWED BY UP TO FIVE DECIMAL DIGITS
DEVICE CODE	D FOLLOWED BY UP TO FIVE DECIMAL DIGITS
SPECIMEN DATE	Y FOLLOWED BY FOUR DIGITS INDICATING A DATE FROM JANUARY 1 (0101) TO DECEMBER 31 (1231)
SPECIMEN TIME	X FOLLOWED BY FOUR DIGITS INDICATING A TIME FROM 0001 TO 2400
OPTION CODE*	O FOLLOWED BY UP TO FIVE DECIMAL DIGITS
EVENT TIME	FOUR DIGITS INDICATING THE TIME OF ENTRY OF THE FIRST DATA ITEM UNDER A GIVEN TEST

*O CODES ARE NOT FILED IN THE DAYFILE

TABLE II.—*Representative Dayfile Sequence*

ENTRY VALUE	ENTRY TYPE	KEYBOARD I. D.
0	A	0
0	T	0
0	S	0
0	D	0
0	Y	0
0	X	0
0	E	0
0	A	1
0	T	1
0	S	1
0	D	1
0	Y	1
0	X	1
0	E	1
22 *	S	1
43401 *	T	1
4101 *	S	0
1115	Y	1
1005	X	1

ENTRY VALUE	ENTRY TYPE	KEYBOARD I. D.
123	A	1
41076 *	T	0
0915	X	0
234	A	0
1102	E	1
7.437	Real Data	1
	Real Data	1
1103	E	0
CO	Ascll Data	0
NT	Ascll Data	0
5.693	Real Data	0
	Real Data	0
2.0	Real Data	0
	Real Data	0
1115	Y	1
1105	X	1
1107	E	1
6.341	Real Data	1
	Real Data	1

* Coded Equivalent

TABLE III.—*Data Directory Entries for T41037*

NAME	CONDITION	DESCRIPTION	UNITS	SEQUENCE
ODHIGH	——	High optical Density	——	1
ODLOW	——	Low Optical Density	——	2
DILUTE	——	Dilution Factor	——	3
SGPT	S	Serum Glutamic Pyruvic Transami	Karmen	4

FILE stores reference information needed by various system processors to perform their job. It is a hash coded file, a filing method which allows reference information to be quickly accessed. Currently this file contains records describing staff members, tests, devices, and calibration data. Animal records are to be added to the file soon. It is beyond the scope of this paper to describe in detail the structure and content of the REFERENCE FILE, but the general content and use of the file should become clear as we describe computer action upon receipt of the various context codes.

DATA DIRECTORY. This file is part of the remote FIS system. It contains detailed specification of each facility test. A test is simply a specific T & E facility job or application which generally results in the acquisition of specific data items. Each such job is assigned a unique test number. The DATA DIRECTORY describes the number of entries in each test, the order of entries, the meaning of each entry and the units of each entry. Table III shows the reference file entries for a typical test. As data for each test is acquired by the Sigma-3, it must correspond exactly with the specifications set down in the DATA DIRECTORY. This is accomplished by generating appropriate displays for the terminal user. The method is illustrated in a later section of this paper.

USER PROCESSORS. As previously indicated, a wide variety of computer processing is handled on the Sigma-3. Required computations may be simple arithmetic operations of a few numbers, as in computation of oxygen transport, or may be relatively lengthy and complex computations as in doing a Fourier analysis of a blood flow or pressure curve. In order for the system to be adequate from the user's point of view, response times at the terminals must be commensurate with the complexity of the process being performed. This means that processors which perform little computation *must not* be delayed even though a lengthy analysis is in the process of being performed. Also, the number of user processors is expected to be continually increasing. In order to allow system growth without continually adding core memory to the system, it is necessary that user processors be resident in core only when being executed.

These capabilities were obtained by adding three semi-resident processing areas, in addition to a non-resident foreground area provided

by the Sigma-3 monitor. Each of these processing areas has its own priority level and an associated queue stack. The queue stacks are used to control orderly loading and execution of multiple requests.

The non-resident foreground area is for the slowest processors. Processors delegated to this level have execution times on the order of minutes. They perform such jobs as communication with the second tier and Fourier analysis of pressure and flow waves. The Sigma-3 monitor handles queuing of this area and controls loading and unloading of the individual processors.

Each of the three semi-resident areas executes at its own distinct priority level. The queuing and loading from the RAD of the non-core processors for these areas are handled by a foreground program. Processing times range from milliseconds for processors assigned to the highest priority semi-resident area to about a minute for processors assigned to the lowest priority semi-resident area.

Whenever a processor is requested and the core area associated with the semi-resident area to which it is assigned is not in use, any lower priority processors are interrupted, the high priority processor is loaded and executed, and then execution of the lower priority processor is continued. The result is a response hierarchy commensurate with the complexity of the jobs being performed.

Using the Terminals

We are now in a position to describe how a user can execute a typical computerized test with the aid of the TEC terminal. We will describe the user's actions and the functioning of the various elements of the computer system as a typical biochemical test is performed. The test we have selected is the test T41037 for Serum Glutamic Pyruvic Transaminase (SGPT) computation. The reference file entries for this test were described in Table III.

When the user arrives at an unused terminal, a message to key in his staff code is displayed (Figure 3). The user keys in his staff code (the keys S, 2, 2 followed by the transmit key) (Figure 4). The computer first checks to see that a staff code has been keyed in. It then checks the

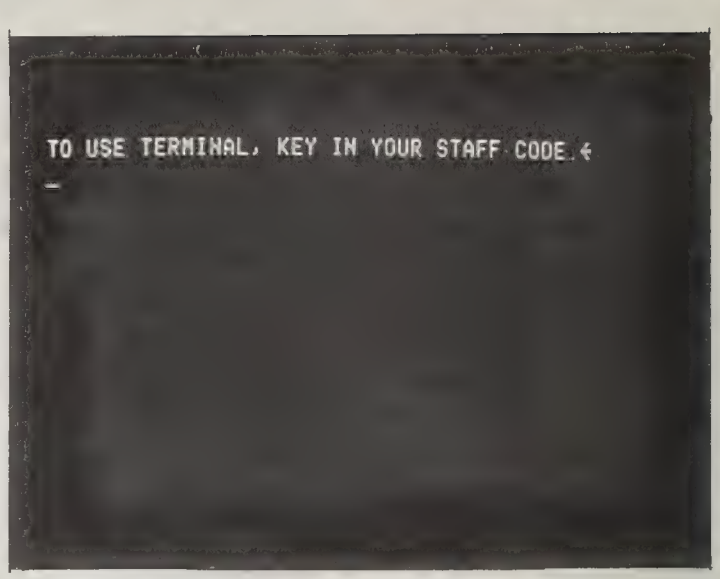


FIGURE 3.—Initial message.

REFERENCE FILE to see if the staff code is in fact entered. If the code is not a staff code, already entered in the REFERENCE FILE, the diagnostic message, INVALID CODE, is generated and the user may reenter a correct staff code. If it is a valid staff code, the message stored in the REFERENCE FILE is displayed on the TEC screen (Figure 5). At the same time the computer stores a computer code corresponding to S22 in the DAYFILE to make this part of the context of any data that follows. The user next keys in the SGPT test number, T41037. (Figure 6). The computer checks the REFERENCE FILE to see if the number T41037 is valid. It then displays the message stored in the

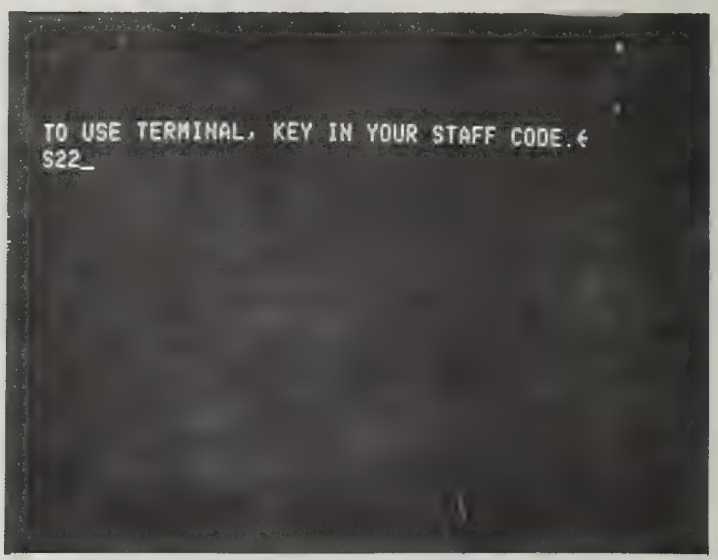


FIGURE 4.—Entering staff code.

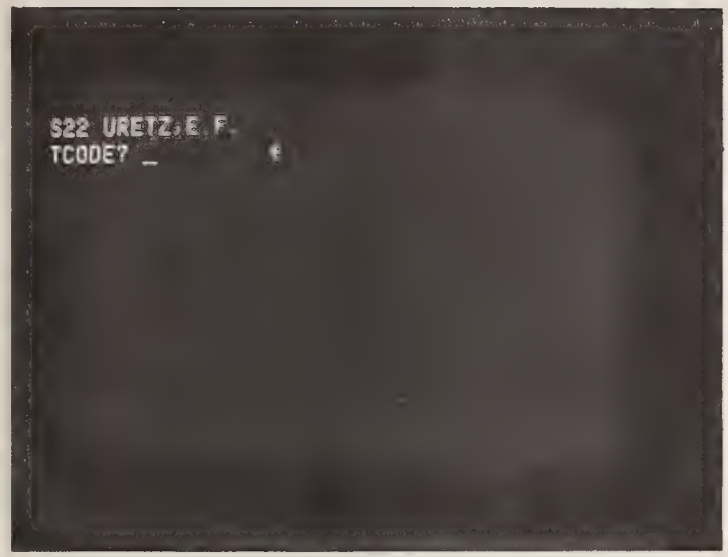


FIGURE 5.—Staff member display.

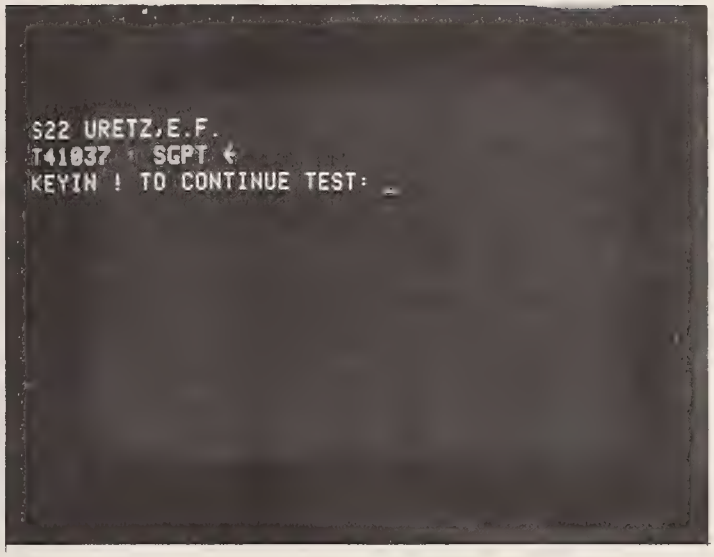


FIGURE 7.—Typical test display.

REFERENCE FILE for T41037 (Figure 7). This confirms that T41037 is the test for obtaining SGPT. The computer also stores zero animal, specimen and device codes in the DAYFILE since a new test is to be executed. It also stores the current data so that the date need not be keyed in if it is current, and, finally, it places a computer version of the code T41037 in the DAYFILE to make this part of the context of any data which follows. The user then follows instructions and keys in an ! (Figure 8). The ! mark is one of the special symbols referred to earlier. It indicates that the computer wishes to execute the test whose context is current for the keyboard, in this case, test T41037.

Each test execution may call for one or more displays (each stored in the REFERENCE FILE) to guide entry of further context and data, for sampling of an analog channel, and for processing of the entered or read data. In the case of test T41037, the display shown in Figure 9 is generated. Notice that the user has been requested to key in certain context codes, required in this test, namely animal code, date and time, as well as a number of data items. Figure 10 shows the display after two of the context codes have been keyed-in and with the third ready to be keyed in. Keying in the A code causes an A with a binary number corresponding to the keyed in number to be stored in the

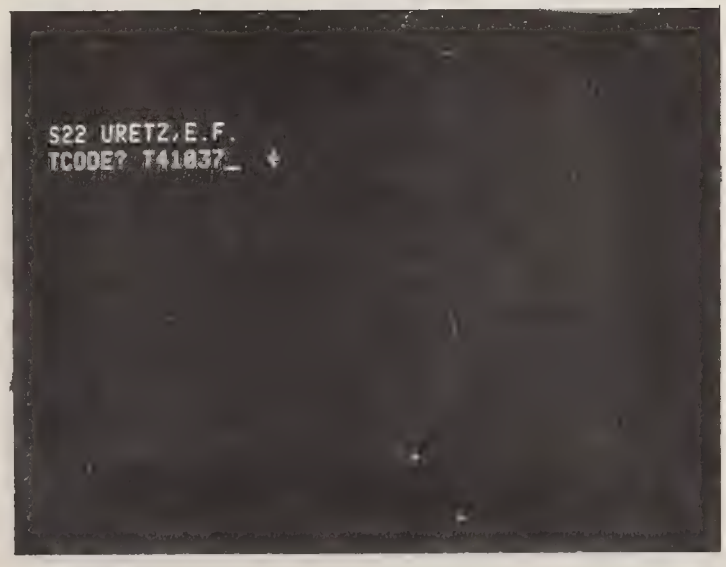


FIGURE 6.—Entering test code.

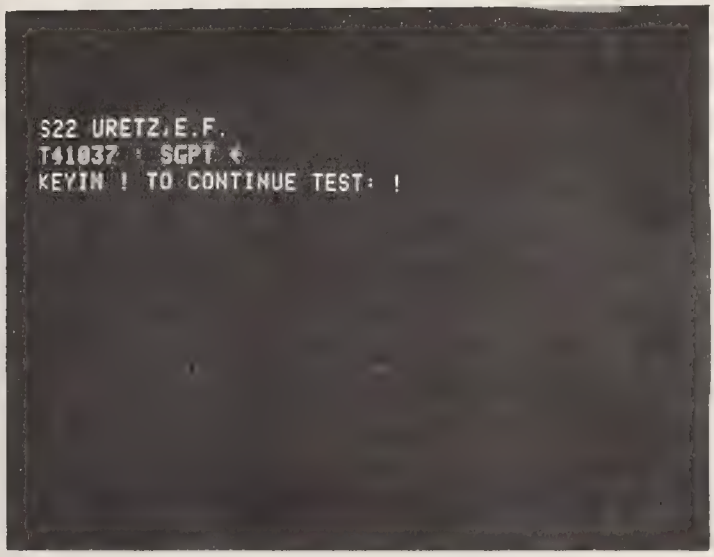


FIGURE 8.—Request for test execution.

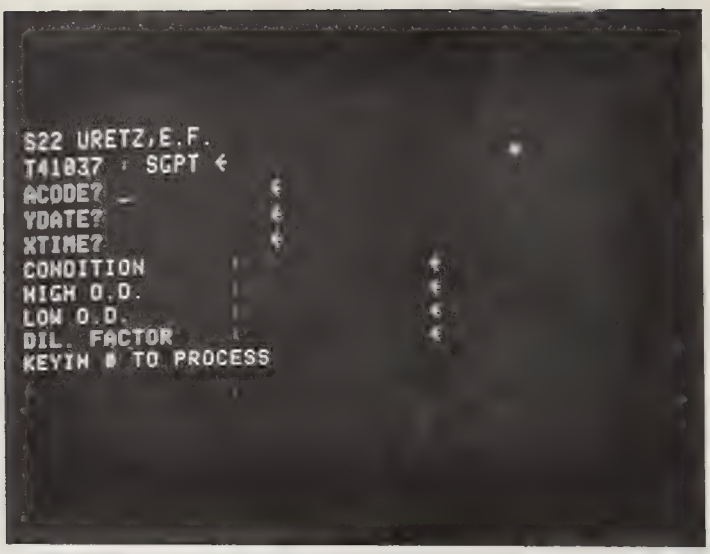


FIGURE 9.—Display to guide test.

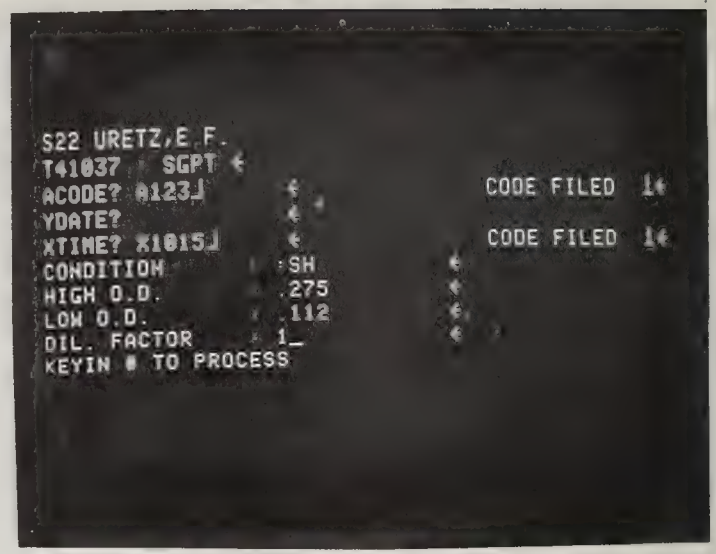


FIGURE 11.—Entering data values.

DAYFILE. It also caused the message CODE FILED to be generated on the same line as the key in. Key in of Y and X causes a similar sequence of events. If the date is current it need not be entered. Next, the user types in the requested data items (Figure 11). Condition is used to request an alphanumeric key-in which is not one of the DATA DIRECTORY entries of the test T41037. It is used in this test to describe any unusual condition of the blood; in this case :SH indicates hemolyzed serum. A colon in front of a sequence of characters indicates alphanumeric data. If no special blood condition exists, this entry may be skipped. The user next uses the 240N spectrophotometer (which is not automated) to obtain a high and low optical density of his sample, types in their values and also indicates the factor by which the sample has been diluted. Notice that although all data items have been typed, none of them have been entered into the computer. The user may examine his typed entries and correct any errors prior to sending the data. He may then transmit all data to the computer by pressing the transmit key. A display indicating that each data item has been received is displayed (Figure 12).

All data items are filed in the DAYFILE. Notice that the three numeric data items corresponds to those in the DATA DIRECTORY

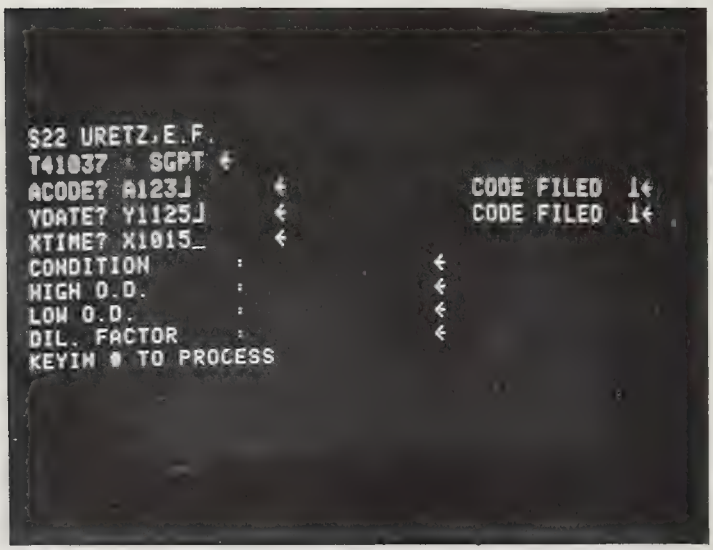


FIGURE 10.—Entering context codes.

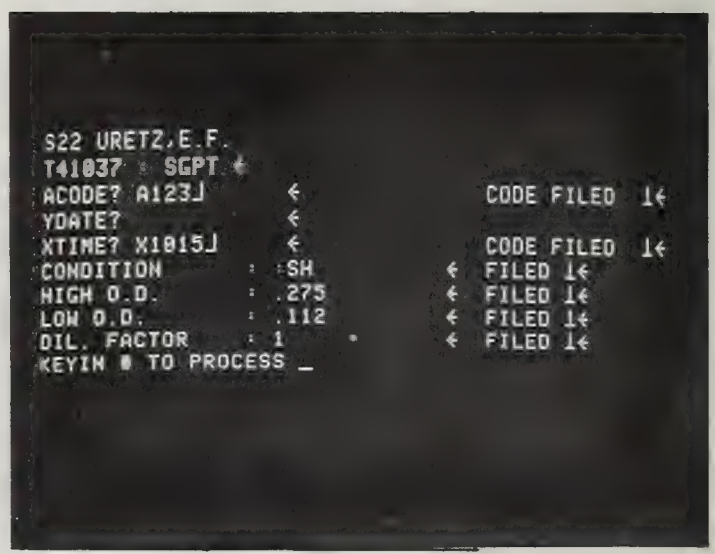


FIGURE 12.—Confirmation of receipt of codes and data.

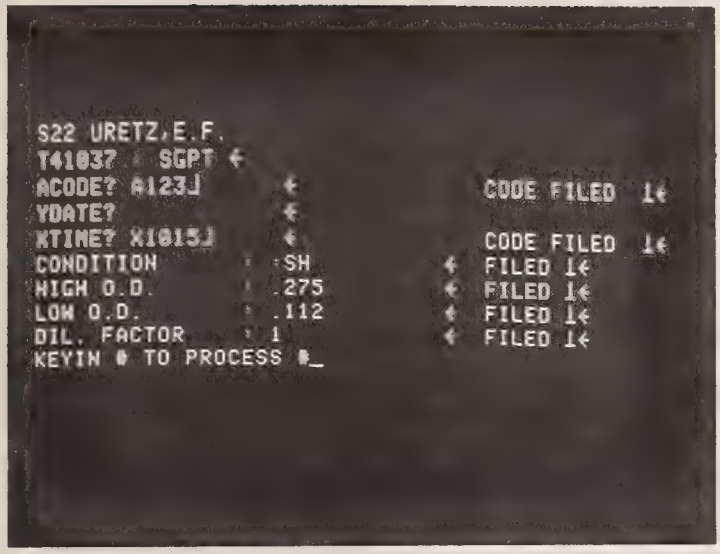


FIGURE 13.—Entering test continuation request.

specification shown in Table III for test T41037. It only remains for the user to key in # as instructed. (Figure 13). This is a special symbol which indicates that the test should be continued. In the case of T41037 this means calling on a processor to perform a simple numeric calculation with the numbers. Since the calculation is a simple one requiring only milliseconds, the T41037 processor has been stored at the highest priority user processor level. This processor is loaded, the processor looks up the keyed in data values in the DAYFILE where they are stored, computes the value of SGPT, stores the computed value in the DAYFILE and generates a display which gives the SGPT result (Figure 14).

The laboratory technician may then key in an ! which causes a new test cycle to start and the display shown in Figure 9 is again generated to guide the next T41037 test. Alternately, he may key in a new test number, or the symbols T9999 which is another special symbol which signs him off the terminal. He may also key in a −2, which is another special symbol. This value is stored in the DAYFILE and instructs the remote system to delete the series of data just entered.

Response Time Considerations and Priority Structure

The priorities to which the various system and user processors have been assigned were carefully selected to give response times commensurate with the complexity of the job being performed. Consider the example of the previous section. The dialogue which occurred between the lab technician and the user was under control of an executive program. This program is expressly designed to handle all dialogues with all terminals essentially simultaneously. Thus, no single terminal is delayed more than a few milliseconds because of dialogues occurring with other terminals. Furthermore, the executive routine as well as terminal reading and writing takes place at a higher priority than all of the user processors, so response to a key in will not be delayed by slow user processors. Also careful assignment of processors to the four priority levels available insures that a fast computation will not be unduly delayed by a slow processor which will be assigned a lower priority. Furthermore, sampling takes place at the highest priority available and will not be delayed by any other foreground or background processing.

Analog Sampling

The example shown above did not require the acquisition of analog data. However, some tests do require sampling and analysis of large amounts of analog data. A SAMPLER program has been developed to accommodate this function. Sampled data is placed in a double buffer

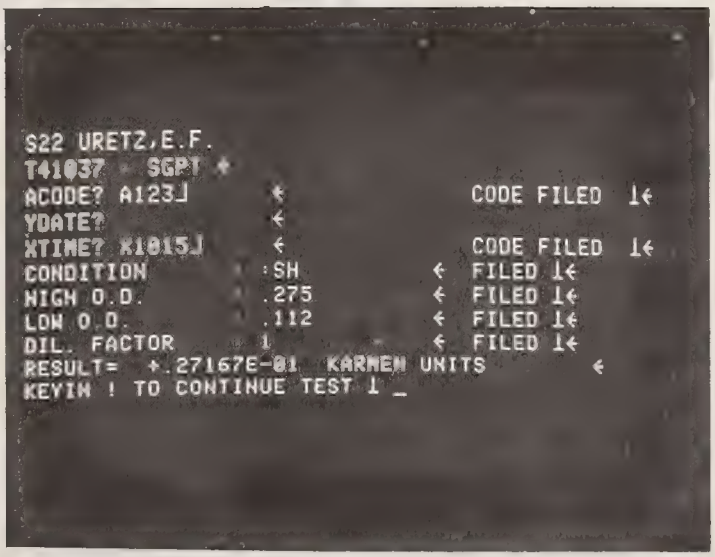


FIGURE 14.—Display of test results.

by the SAMPLER and as each buffer is filled it is automatically read to an area on the RAD temporarily assigned for this purpose. Twelve such areas exist, each able to hold 3,060 words of data. This is sufficient to store six seconds of data sampled at the highest rate available to a user (500 samples/second). When sampling is required by a test, key-in of the # will cause the sampling to be performed prior to loading of the test processor. The processor then retrieves data from the RAD, processes it, places results in the DAYFILE and then releases the RAD area for use by other processors.

Status of the System

The terminal system has been designed to handle biochemical, electronic, and physiological data acquisition and processing. To date, most biochemical data processing has been converted to the terminal system. Electronic and physiological data acquisition and processing have not yet been added to the terminal system, although some preliminary work in the area of physiological monitoring has been conducted. The physiological monitoring had as its goal computation of A.C. peripheral and pulmonary resistance of a calf. Two analog channels of data were sampled at a 500 per second rate for five seconds. One channel monitored pressure and the second channel monitored flow. Each channel was calibrated at three values, and linear regression analysis was used to obtain calibration curves for each signal. A peak picking algorithm was used to detect signal cycles, and Fourier analysis was used to obtain ten harmonics of each wave. The harmonics were then used to compute peripheral or pulmonary resistance of the calf.

A list of the tests currently implemented on the Sigma-3 is given in Table IV. These tests fall into several categories. In one category are the tests used strictly to key in data and their identifiers. These are the tests which require neither instrument reading or data processing.

In another category are the tests which use the Gilford 300N to obtain optical density of a sample. These tests generally require calibration data to be prestored in the REFERENCE FILE either daily or bi-weekly. The stored calibration data is stored as a series of line segments which approximate the measured calibration data. The Gilford processor obtains its result by using the calibration data to interpolate the test variable value from the read value of optical density.

A third category of tests includes those which read no instrument but which compute results from keyed-in data values or data stored in the DAYFILE by another test. Thus, the Albumin and A/G ratio test obtain their results by looking up Total Protein and Globulin of each specimen in the DAYFILE and performing simple arithmetic operations on this data. Another example, is the Serum Glutamic Pyruvic Transami test described earlier in which the computer calculates SGPT from keyed in values of optical density.

A more complex test is the Lactate Dehydrogenase Isoenzymes test which reads the densitometer for 60 seconds at a rate of 10 samples per second. The six hundred points are sampled and stored on the RAD and then the densitometer processor is called. The processor finds the five distinct waves in the densitometer data corresponding to LDH isoenzymes, integrates each wave to obtain its area, and computes the ratio of each area to the total curve area.

Automated tests which require careful coordination between lab technician and the user are the red and white blood count tests. In these tests, the coulter counter is used repeatedly until three readings agree to within 10%, and then the average of the agreeing readings is taken and a correction factor applied to obtain the corrected blood count. The average and corrected counts are stored in the DAYFILE and displayed on the terminal. Each time the computer is instructed to read the Coulter counter, it prints the current and previous two readings of the counter and informs the lab worker of the test status.

SUMMARY

We have described a terminal data acquisition system used to acquire laboratory data and to aid scientific workers in performing experiments. The features of the system which make

TABLE IV.—*Tests Implemented on the Sigma–3*

TABLE 4 - TESTS IMPLEMENTED ON THE SIGMA-3

TEST NAME	DIRECT READ INSTRUMENT	PROCESSOR
Device Log Inn	None	Reference File
Test Calibration Entry	None	Reference File
Hematocrit	None	None
Red Blood Count	Coulter Counter	Coulter
White Blood Count	Coulter Counter	Coulter
Platelet	None	None
Differentials	None	None
Prothrombin Time	None	None
Clot Time	None	None
Plasma Hemoglobin	Gilford 300 N	Gilford

it a versatile and powerful tool include the following:

1. The ability to service multiple users simultaneously, with response times to each user commensurate with the complexity of the task being performed.
2. The ability to sample multiple analog channels at rates up to 500 samples per second with integrity, no matter how much foreground or background computation is in progress.
3. The ability to store data from multiple experiments or tests with sufficient descriptors to uniquely identify all such data. The data is transmitted once a day to the Univac 1108 FIS system where it is integrated into more organized and comprehensive files and, thereby, made available for extensive statistical and mathematical analysis.
4. The ability to readily accommodate new experiments or laboratory tests with mini-

TABLE IV.—*Tests Implemented on the Sigma-3*—Continued

TABLE 4 - CONTINUED

Lactate Dehydrogenase Isoenzymes	Densitometer	Densitometer
Creatinine	Gilford 300 N	Gilford
Lactate Dehydrogenose	None	General
Urea Nitrogen	Gilford 300 N	Gilford
Glucose	Gilford 300 N	Gilford
Phosphorus	Gilford 300 N	Gilford
Amylase	Gilford 300 N	Gilford
Acid Phosphatase	Gilford 300 N	Gilford
Alkaline Phosphatase	Gilford 300 N	Gilford
Hemoglobin	Gilford 300 N	Gilford
Oxygenator	None	Oxygenator

mum disruption of those already implemented.

DISCUSSION

N. P. CHING, St. Vincent's Hospital, New York City: It seems as though your system is really the routine hospital lab computer. If I were doing, say a dog or we had a patient being monitored over a one hour period, can your system handle the various variables, like blood gas, cardiac output, etc.?

MR. URETZ: Yes, basically our processors are called as needed. You would never sit with a program in for an hour. We're in the process of doing the equivalent of what Sam Topham was talking about for routine long-term monitoring. This means that we will be building additional software so that periodically the appropriate program gets called upon and loaded-in to do the job. We've gone about this in the other way, going to the lab data first. We have on some demand physiological measurements and later we will add this other capability. But the basics are there. It's just a new structure to be built on top.

TABLE IV.—*Tests Implemented on the Sigma-3*—Continued

TABLE 4 - CONTINUED

Partial Thrombinplastin Time	None	None
Lactate	None	Lactate
Cholesterol	Gilford 300 N	Gilford
Serum Glutamic Oxalacetric Transami	None	General
Serum Glutamic Pyruvic Transami	None	General
Total Bilirubin	Gilford 300 N	Gilford
Direct Bilirubin	Gilford 300 N	Gilford
Total Protein	Gilford 300 N	Gilford
Globulin	Gilford 300 N	Gilford
Albumin & A G Ratio	None	Albumin

DR. CHING: What kind of a load can you handle, in addition to this other, numerically?

MR. URETZ: When you say a load I'm not quite clear what you mean?

DR. CHING: Well, if we have the patient being monitored continuously and then for specific moments during the day, can we call it in?

MR. URETZ: Yes.

DR. CHING: Is there a limit to the number of times we could call it in?

MR. URETZ: No. there would be a limit to the number of channels you can monitor at one time. We have a maximum sampling rate of 500 samples per second and we can do about ten channels at that rate. If you go down to 100 samples per second, you can be monitoring about 50 channels. And probably while it's going on, all these other tests will be handled with no more than perhaps a slight sluggishness, if you had a tremendous number of terminals. The main thing that could slow you down is to sample to many channels to fast.

CHAIRMAN WARNER: The thing I wonder about is, since you have identical hardware, are you going to be able to share any of your efforts? It looks to me like you've got completely different file structures and a completely different monitor system.

MR. URETZ: Right. The basic computer monitor is the same, but the executives are different. I think the sharing will come at the level of the user programs which, with a little doctoring, can be made to fit. Once all the problems of this system design are out of the way and things are running, I think it's then a matter of working with the basic processors themselves and sharing those. Data, once it is ours, goes to the second tier, where it can be organized in almost any form. It's a very versatile thing and also tapes of data can be handed over for comparative work. But no, we'll never take a program verbatim from one system and just routinely put it on the other. But the concept and much of the programming can be used.

CHAIRMAN: One thing that wasn't clear to me is, are you sharing core? Do you have multiple programs in core at the same time or are you doing everything with swapping?

MR. URETZ: We are not swapping. All the executive tasks, the "talking" of the terminals to the user, is core-sident executive and practically instantaneous and simultaneous. The user processors are only called for that split second of time when they're needed. Then they're loaded in four areas available with from 1,000 to 1,500 words. One is really the backgound area with 8,000 words and the processor is called only after all sampling has been completed. After all the dialogue has taken place and when a processor is called, it goes to the reference file to get the calibration data information. It goes to the day file which stores data to get any data it needs. These things can be done very quickly because, in general, the data you want is still in core, it hasn't gone up to the disc. We have two sectors where there are 360 words worth in core at all times and the process is carried out and the program executed. If a program is long, e.g., execution takes a minute, it gets into priority number, say, three, with other programs of that order of time and there's a stack kept. So a response to a user might get slowed down if three or four users want one minute processors. But the milliseconds-per-use processor, which is the majority to date of processor I would say, gets in and out so fast (and ahead of the very long processors) that you get good response time for all users.

CHAIRMAN: The UNIVAC, you said, is not a time-sharing system, so you're just batching it over to UNIVAC periodically? Or are you sending it over as it's accumulated?

MR. URETZ: We send the data over once a day. We have some summary capabilities. We haven't put these in now. Right now, other than to give user's results for a particular test, if he wants to see some summary data over any period of time, he has to wait until we send the data specifically. Now, if he's real anxious, we can at any time initiate this, send the day file, and continue from there. But generally, we send it once a day and for the kind of operation we're running, this seems to be fine. People generally aren't trying to do summary statistical work on-line. At least they haven't so far.

THE CONSCIOUS CALF PREPARATION: TECHNICAL ASPECTS OF SURGERY, ANIMAL CARE, IMPLANTED INSTRUMENTATION AND MONITORING FOR CARDIOVASCULAR DATA ACQUISITION

K. T. Weber, J. S. Janicki, A. A. Walker* and B. H. Dennison**

Only recently has the calf been used with any frequency in the experimental cardiovascular laboratory. This growing popularity is in large measure due to the following: (1) the animal's docility for chronic experimentation, (2) its suitability for thoracic surgery, (3) availability, and (4) the larger size which greatly enhances its usage for a number of physiological and anatomical considerations. This presentation is primarily a description of the technical, as well as practical, aspects of the chronic calf preparation. Particular emphasis is placed on the interfacing of the animal and data acquisition system, the data monitoring equipment itself, and pre- and post-operative care. In addition, hemodynamic measurements obtained in unanesthetized, resting calves and various applications of the preparation are discussed.

INTRODUCTION***

In the cardiovascular laboratory, the calf has become increasingly popular as an experimental animal. This is due in large measure to its cardiovascular size and capacity, and to the fact that the major vessels are easily accessible and capable of withstanding the trauma of chronic instrumentation. Other desirable qualities are its docility, which simplifies the task of data collection in the conscious state, and availability. Experience with other large animals was less encouraging. Large dogs (>60 lbs.) required conditioning and training; furthermore, they have a high incidence of Dirofilaria immitis (heartworms). In sheep, extreme myocardial irritability and ventricular fibrillation often occurred during manipulation of the heart. For chronic data collection, the pig was intractible.

The majority of our experience with calves (over 100 animals) was gained while at the National Heart and Lung Institute and in conjunction with Meloy Laboratories. Recently, we have initiated these experiments in our laboratory here at the University of Alabama Medical Center.

The purpose of this presentation is to describe in detail various aspects of our experimentation with the fully-instrumented, conscious calf preparation. In particular, the surgery, animal care, components of the total data monitoring system and the various applications of this preparation are discussed.

METHODS

General

Holstein, Hereford and Charolais calves, 4 to 12 weeks of age and weighing 50–100 kg, have been utilized in the majority of cases. The Angus has been less suitable for our purposes because of the difficulty in cannulating the femoral artery which is located deep and posteriomedially. In addition, the short thigh does not permit adequate extension and often leads to cannula kinking. The calves are housed (both pre- and post-operatively) in portable metabolic cages* or 3′ x 4′ stalls with stanchions. We personally perfer the cage because of its ease in transporting the animal, as well as its suitability for maintaining an overall cleaner environment for the post-operative animal. In this regard, we have modified the floor of the cage to include a sliding pan for the collection of excreta. The animals are given grain and water pre-operatively (alfalfa has been used ad libetum) until 24 to 48 hours prior to surgery when

* Department of Medicine, University of Alabama, Birmingham, Alabama.

** Meloy Laboratories, Springfield, Virginia.

*** The research upon which this publication is based was performed pursuant to the Medical Devices Applications Program Contract PH 43-67-1418 and N.I.H. Grant HE 11,310, National Institutes of Health.

* Humane Equipment Corporation, Baraboo, Wisconsin

food is discontinued. Water fast begins at 12 to 24 hours.

Anesthesia and Surgery

We have recently chosen intravenous ketamine[1] (Ketalar)** for the induction (12-16 mg/kg) and maintenance (6-8 mg/kg given slowly every 15–20 minutes) of anesthesia. Succinyl choline (100 mgm) is given together with the initial ketamine dose to facilitate endotracheal intubation since the latter drug preserves pharyngeal reflexes. Using ketamine, induction was seen within 30–40 seconds and the long post-operative sedation period inherent with the use of barbituates (12 hours or more), fluothane or fluothane and nitrous oxide mixtures (3–4 hours) was avoided. Ketamine has also negated the need for additional and expensive anesthesia equipment, such as absorber circuits and a recirculation apparatus which are necessary with volatile anesthetics. In addition, an anesthetist is not required. To date, hypotension or respiratory depression has not been observed with this agent.

Endotrachael intubation was accomplished with a Size 44F endotrachael tube and a long bladed laryngoscope. Adequate cuff inflation was checked by manual compression of the lower thoracic cage and diaphragm. Ventilation was maintained via a pressure cycled respirator (Bird Mark VII or VIII) at an average inspiratory pressure of 20–22 cm of water (range 18–30). Compressed air or various oxygen-air mixtures was adequate for these purposes. Periodic sighing was utilized during thoractomy and closure to minimize atelectasis. Blood pO_2, pCO_2 and pH were checked throughout the operative procedure to aid in their maintenance in the physiological range.[2]

Succinylcholine (Anectine)* blockade was used during the incision of the thoracic musculature. An intravenous bolus injection (40 mg) or drip (600 mg/500 cc saline) has been found adequate for these purposes.

Pre-operative hematocrit and central venous pressure via the jugular vein were obtained as a guide to subsequent fluid replacement. A liter of normal saline or Ringer's lactate containing 5 million units of penicillin was routinely given during each case. Blood replacement was rarely necessary; however, when needed, unmatched donor blood from adult cattle has been used without reaction.

In the right recumbent position with the neck and left chest shaved and prepped using standard procedures, the jugular vein was isolated and cannulated and the chest entered through the fifth intercostal space. When more extensive exposure was necessary, the fifth rib was resected. The toleration to resection has been quite satisfactory. Once in the chest, the pericardium is incised and the following instrumentation implanted. The specific characteristics and care of each device will be discussed subsequently.

Instrumentation (see Figure 1). (a) A Teflon catheter (#8F with length fixed at surgery) was inserted via the internal mammary artery and advanced to the central aortic region (bra-

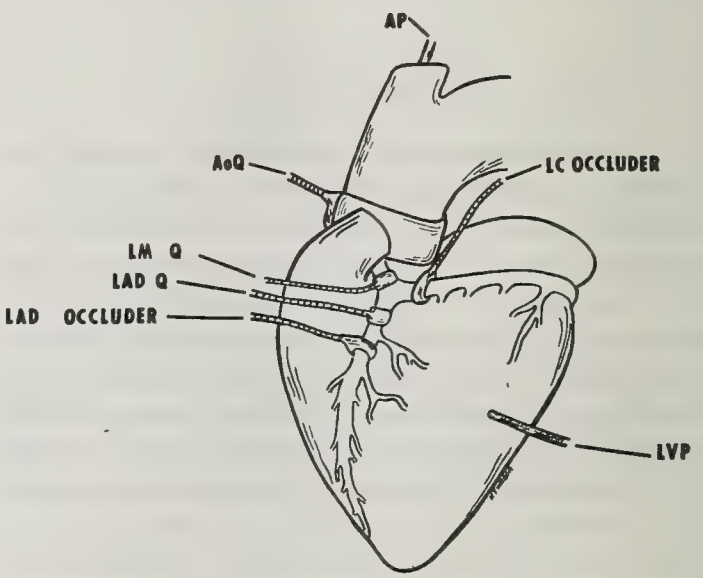


FIGURE 1.—Diagrammatic representation of instrumented calf heart. Included are aortic root flow probe (AoQ); left main (LM) and left anterior descending (LAD) flow probes; and left anterior descending and left circumflex (LC) occluders. In addition, left ventricular pressure transducer cable (LFP), and brachiocephalic pressure catheter (AP) are shown. (Printed with permission of the author from "Left ventricular failure and cardiogenic shock following acute myocardial infarction: A correlation of clinical and experimental observations." *Conference on Research Animals in Medicine*, 1972.)

* Burroughs Wellcome.
** Parke-Davis, Detroit, Michigan.

chiocephalic artery) for arterial pressure monitoring. (b) A pressure transducer*,** was implanted into the left ventricular chamber via either the apex or through an avascular area of the anterior epicardial surface (posterior to the anterior papillary muscle) and secured in place with a purse-string suture. With the apical approach, the transducer and a one-inch segment of the lead wire floated freely within the left ventricular chamber, whereas with the higher approach, the transducer was placed flush against the endocardial surface to minimize possible papillary muscle artifact. (c) An electromagnetic flow probe*,** was placed around the aortic root (20–26mm diameter). (d) Epicardial ECG leads were sewn to the heart using stainless steel suture. These may also be used for pacing. Direct myocardial leads were chosen because of the difficulty in using and securing limb leads in the conscious animal. (e) For coronary sinus blood sampling, the sinus was cannulated via the hemiazygous vein which, in the calf, joins the coronary sinus approximately 5cm proximal to its termination into the right atrium. The hemiazygous may be ligated without side effects. (f) Other devices representing particular needs of the protocol have also been utilized. The latter include vascular occluders*** positioned around the coronary arteries, an intra-aortic balloon (Tecna Corporation, Emeryville, Calif.) inserted into the descending thoracic aorta via the femoral artery, and other assist devices which have been previously reported.[3] The surgical instrumentation necessary for these procedures is, in large measure, similar to those available in most laboratories using dogs.

Following instrumentation, the chest cavity was thoroughly rinsed with warmed saline containing 10 million units of penicillin and aspirated. Each lead wire was brought out, in a staggered fashion, through the original incision, and secured in place with a purse-string suture. Divided muscle is carefully approximated and closed with continuous suture. Skin is closed with mattress suture (00 silk) to prevent wound dehiscence. Lead wires were secured in place externally with skin suture. All catheters were capped with Luer locks, filled with pure heparin and patency checked daily. Systemic anticoagulation was only needed 24–48 hours following surgery with this technique. A loose cloth binder is placed around the thorax to prevent damage to instrument leads. A chest tube for fluid drainage was placed in the seventh intercostal space and withdrawn 24 hours post-operatively. Vacuum on the chest line was kept at 10 cm H_2O or less negative pressure. Fluid loss (primarily serous) during this period was approximately one liter.

Post-Operative Care

The animals were maintained on intramuscular penicillin (1.2 million units, two to four times daily) for fourteen days regardless of body temperature (rectal temperature of 101–102° may be considered in the normal range). Intravenous fluids were continued as needed; however, this has not usually been necessary beyond the first 24 hours post-operatively. Electrolyte abnormalities were a rarity. Food and water were provided ad libetum post-operatively; however, no more than one-half bucket of grain for the first 24 hours should be provided to avoid bloating, thus compromising ventilatory exchange. The animals were generally standing within two hours following surgery (ketamine anesthesia), although their condition was still quite weak. Viral pneumonias are occasionally seen during the post-operative period and have been unsuccessfully treated with presently available antibiotic regimens. The incidence of pneumonia appears to be higher in animals obtained through auctions, where exposure to a larger number and variety of pathogens occur. All post-operative calves were kept in a separate room in an attempt to minimize wound infections. Subcutaneous infections which do occur are generally well loculated and tolerated by the animal. However, as an adjunct to treatment, Streptokinase-streptodornase (Varidase)* and drainage are occasionally used. The room and interfacing with the data acquisition system will be described later. Ultimate

* Konigsberg Institute, Pasadena, California.
** DynaSciences, Pasadena, California.
* In Vivo Metrics, Redwood Valley, California.
** Biotronex Laboratory, Inc., Bethesda, Maryland.
*** Rhodes Medical Instruments, Woodland Hills, California.

* Lederle Laboratories, Pearl River, New York.

survival was determined by the needs of the particular experimental protocol. A routine autopsy was performed and, in addition to the relevant pathologic examination, a careful examination of instrument location and position was determined.

Instrumentation

We have found that frequent manipulation of the lead wires serves to condition and acclimate the animal to the data monitoring process. Irritation or discomfort to the animal was rare during data collection with a two to seven day recovery period. In some cases, additional time was necessary until the animal was symptom free. At no time were sedatives necessary.

Specific care and maintenance of the implantable pressure transducers, arterial catheters and flow probes has been previously reported.[4] All hardware was sterilized with ethylene oxide. Cold sterilization with benzalkonium chloride (Zephiran) has also been used on occasion. The arterial catheters were filled with pure heparin. Recently, in an attempt to further minimize thrombus formation, the catheter lumen has been coated with colloidal graphite* as described by Folts.[5] Arterial pressure was monitored with a Statham P23Gb transducer positioned at heart level and zeroed. The left ventricular implantable pressure transducer likewise was coated with colloidal graphite to minimize thrombogenecity and tissue overgrowth. The transducer was calibrated prior to surgery by a technique developed by Dennison.[4] Briefly, the basic calibration procedure was carried out in water at 37° over a pressure range of 0–200 mmHg. In an attempt to reduce errors inherent with this type of transducer, we observe the following rules: (a) excitation at approximately 50% of rated voltage,[6] (b) utilization of low-drift compensation resistors necessary only with the DynaSciences model, (c) maintenance of a performance log with rejection of all transducers demonstrating significant drift, and (d) careful matching of central aortic and left ventricular systolic pressure to compensate for zero drift. Other techniques which were used to optimize performance have been discussed under the surgical section.

* Acheson Colloid Company, Port Huron, Michigan

The vascular occluders consist of an inflatable cuff and length of tubing which were filled with mercury instead of air or saline. This method was chosen in order to minimize stress-relaxation of the balloon and uneven inflation seen when air alone was used. We feel that occlusiveness may also be more carefully controlled with this technique. Left main coronary flow is monitored during the occlusion as an aid in quantifying reductions in flow.

All data were recorded at the post-operative animal location on a multichannel paper recorder* (see Figures 2 and 3). This serves for establishing baselines and zeroes, as well as for monitoring basal states and responses to various diagnostic and therapeutic interventions. All data were simultaneously recorded via cable at a distal site on analog tape and converted at a rate of 250 samples/second to digital form using the PCP7 computer. The animal may be left alone while connected to the recording instrument in order to provide a stress-free, quiet environment. The analog tape is later edited for additional and more specific derivations which are enumerated in the hemodynamic section below.

The total data monitoring system thus includes the implanted instrumentation in the biological system, the paper recorder for immediate assessment of baselines and specific re-

* Hewlett Packard

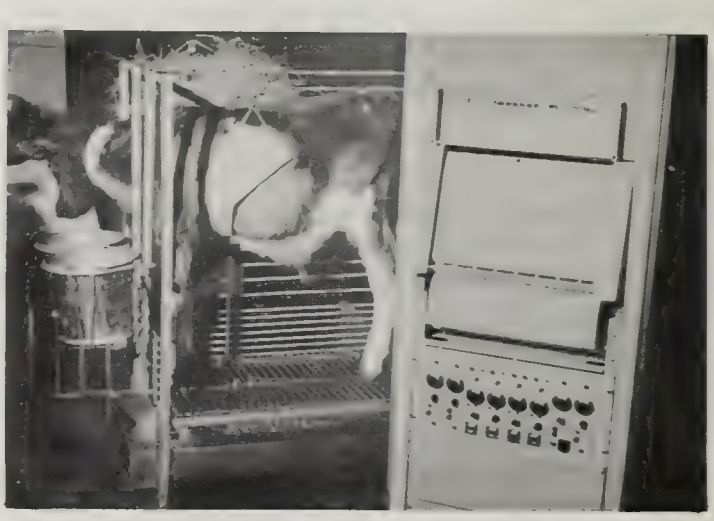


FIGURE 2.—Post-operative, instrumented animal in metabolic cage. Paper recorder for monitoring also shown.

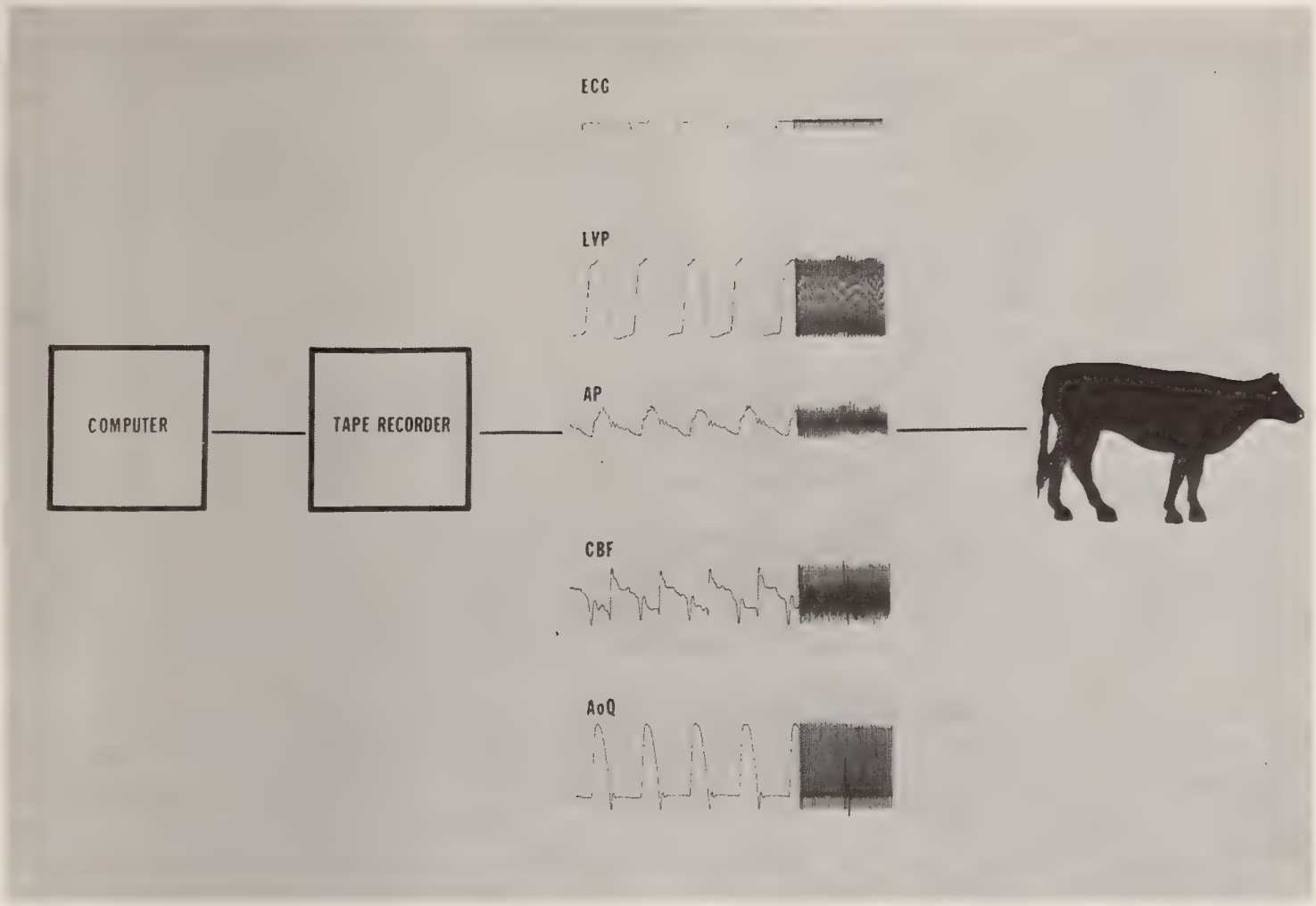


FIGURE 3.—Diagrammatic representation of data monitoring system, including the calf, paper recorder, tape recorder and computer.

sponses, the analog tape and the digital computer facilities (see Figure 3).

Hemodynamics

The following may be derived from the recorded data using digital and analog techniques: peak, mean and integrated aortic flow and pressures; systolic time intervals including electromechanical systole or the QS_2 period, pre-ejection period, and left ventricular ejection time; the first derivative of left ventricular pressure; mean ejection rate; tension time index per beat and per minute; cardiac stroke and minute work and cardiac output.

RESULTS AND DISCUSSION

Baseline Hemodynamics

Fourteen calves as previously reported[4] were utilized for the assessment of baseline hemodynamics in the resting, conscious state. The data and results obtained are presented in Tables I and II and Figure 4 and include values for left ventricular pressure, maximum rate of pressure rise, pulse pressure, heart rate, stroke volume, cardiac output and their respective indices, mean ejection rate, stroke work and power, and systolic and diastolic time intervals. Similar studies by others[7] have been hampered by the need for restraint-tables and sedation.

Ischemic Heart Model

Experimental myocardial ischemia and infarction has been studied as previously reported[8-10] in over 30 calves both acutely and chronically. Diffuse myocardial microcirculatory ischemia, of varying severity (dosage), has been created using microspheres 6–14 micron in diameter. The microspheres were in-

TABLE I.—*Hemodynamic Data**

Animal	LVP	MRPR	AP	HR	SV	SI	CO	CI	MER	SW	SP
1	150/7	1800	150/110	95	81	38	7.7	3.6	316	141	630
2	140/10	1900	140/87	91	82	38	7.5	3.5	350	128	547
3	140/10	1640	140/95	92	85	40	7.8	3.6	362	129	549
4	132/8	1600	132/96	90	87	41	7.8	3.6	350	130	528
5	125/10	2200	125/85	120	79	37	9.5	4.4	411	115	599
6	140/10	1800	140/105	96	91	43	9.6	4.5	437	151	726
7	135/9	2000	135/105	129	74	35	9.5	4.4	451	109	664
8	145/8	1800	145/110	100	84	39	8.4	3.9	388	140	648
9	118/8	1560	118/75	100	82	38	8.2	3.8	347	114	483
10	110/4	1600	110/75	93	83	39	7.7	3.6	352	111	444
11	150/8	2200	150/100	129	72	34	9.3	4.3	378	125	657
12	135/9	2000	135/100	120	78	36	9.4	4.4	379	125	607
13	140/10	2080	140/100	80	96	45	7.7	3.6	384	163	653
14	118/8	1500	118/68	78	101	47	7.9	3.7	363	137	494
Mean	135/9	1719	134/94	101	84	40	8.4	3.9	380	130	588
Range	110/150	1560–2200	110–140	78–129	72–101	34–47	7.5–9.6	3.5–4.5	350–451	109–163	444–664

* Data derived from 14 calves in the basal standing state.

Abbreviations: LVP = left ventricular pressure (peak systolic/end diastolic) in mm Hg; MRPR = maximum rate of pressure rise in mm Hg/sec; AP = arterial pressure in mm Hg; HR = heart rate in beats/min; SV = stroke volume in cc; SI = stroke index in cc/m^2; CO = cardiac output in liters/min; CI = cardiac index in liters/min/m^2; MER = mean ejection rate in cc/sec; SW = stroke work in gram meters; and SP = stroke power in gram meters/sec. (*J. Surg. Res.* XI: 383–389, 1971. Printed by permission of the author.)

fused over a period of minutes or hours into the left main coronary artery. In addition to the basic hemodynamic measurements and other calculated variables, angiotensin stress responses (2.5 mgm/500 cc saline) were also evaluated following infarction (Figure 5).

Acute coronary occlusion using vascular occluders has been evaluated in a number of experiments.[9-10] An example of the change in hemodynamics following occlusion is shown in Figure 6. Our experience with premature ventricuar contractions (PVC's, unifocal) and subsequent ventricular tachycardia and fibrillation (VF) following occlusion was similar to observations in dogs.[11] Generally, the PVC's occur within 2 to 15 minutes of occlusion and progress rapidly (10 minutes) to VF. Our success rate in defibrillating the heart using routine cardioresuscitative efforts has been quite low (2–5%) and is similar to the experience of Donawick. Recently, however, we have been able, with the use of massive doses of regular insulin (400–1200 units)*,** given as a bolus, intravenous injection every two to five minutes, to not only control or abate PVC's and ventricular tachycardia, but to proceed with multiple occlusions for controllable, more severe levels of left ventricular dysfunction. We are presently investigating these findings in more detail.

TABLE II.—*Measured Systolic and Diastolic Time Intervals**

Animal	HR	QS_2	LVET	PEP	Q-Q	D	QS_2/D
1	95	328	224	104	627	299	1.09
2	91	334	234	81	664	329	1.01
3	92	348	235	113	656	308	1.12
4	90	325	246	78	675	350	0.93
5	120	256	192	64	500	244	1.05
6	96	300	208	92	617	317	0.95
7	129	245	164	81	456	211	1.16
8	100	294	216	78	600	305	0.96
9	100	315	236	79	595	280	1.12
10	93	328	250	78	643	315	1.04
11	129	248	190	58	456	208	1.19
12	120	282	206	75	496	214	1.31
13	80	352	250	102	706	354	0.99
14	78	360	278	82	770	410	0.83
Mean	102	309	222	85	605	294	1.05
Range	78–129	245–360	164–278	58–113	456–770	211–410	0.83–1.19

* Data derived from 14 calves in the basal state. All time periods are expressed in milliseconds.

Abbreviations: HR = heart rate in beats/min; QS_2 = electromechanical systole; LVET = left ventricular ejection time; PEP = preejection period; Q-Q = the Q-Q interval of the eletrocardiogram; D = duration of diastole; QS_2/D = the rate of systole to diastole. (*J. Surg. Res.* XI: 383, 1971. Printed by permission of the author.)

Circulatory Assist

Another example of the utility of this preparation was the chronic implantation and hemodynamic assessment of the intra-aortic balloon.

* Eli Lilly and Company, Indianapolis, Indiana.

** E. R. Squibb & Sons, New York, New York.

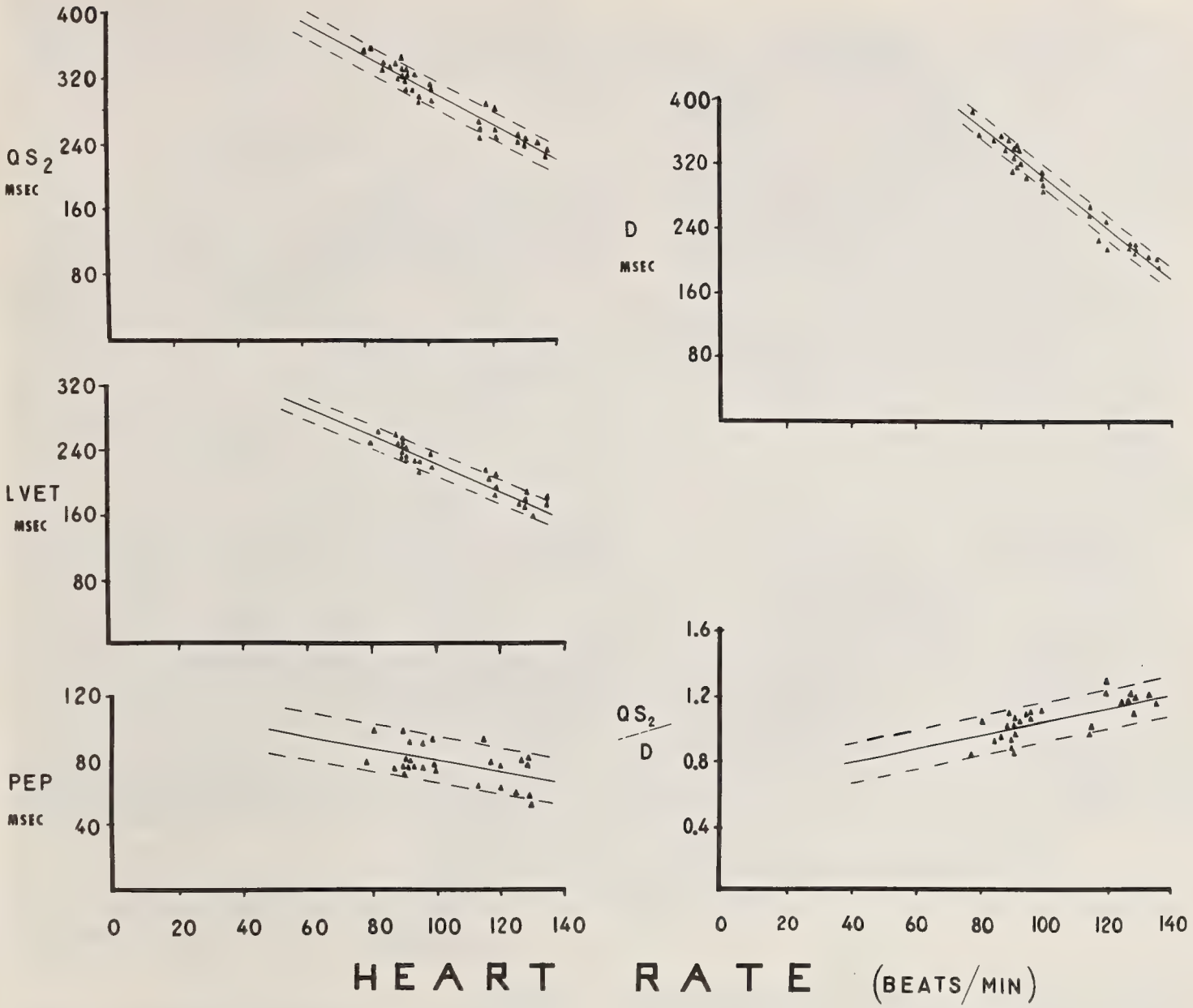


FIGURE 4.—Regression lines for systolic time intervals at various heart rates. Electromechanical systole (QS_2); left ventricular ejection period (LVET); the preejection period (PEP); diastole (D); and the ratio of systole to diastole (QS_2/D) derived from 14 calves are plotted (in milliseconds) against the normal regression line (± 1 SD) and heart rate. (J. Surg. Res. XI:383–389. 1971. Printed with permission of the author.)

The importance of inflation-deflation duration or timing and balloon position on intra-aortic balloon pumping performance has been reported (see Figure 7).[13] Studies evaluating pumping effectiveness during induced coronary insufficiency have also been initiated.

SUMMARY

The feasibility of an instrumented conscious calf preparation has been demonstrated. We have emphasized its application to cardiovascular data acquisition and problem solving. Its potential usefulness in other areas, however, remains to be determined. We have presented our experimentation and data monitoring in some detail so that others might better utilize the preparation in pursuing other problems of interest.

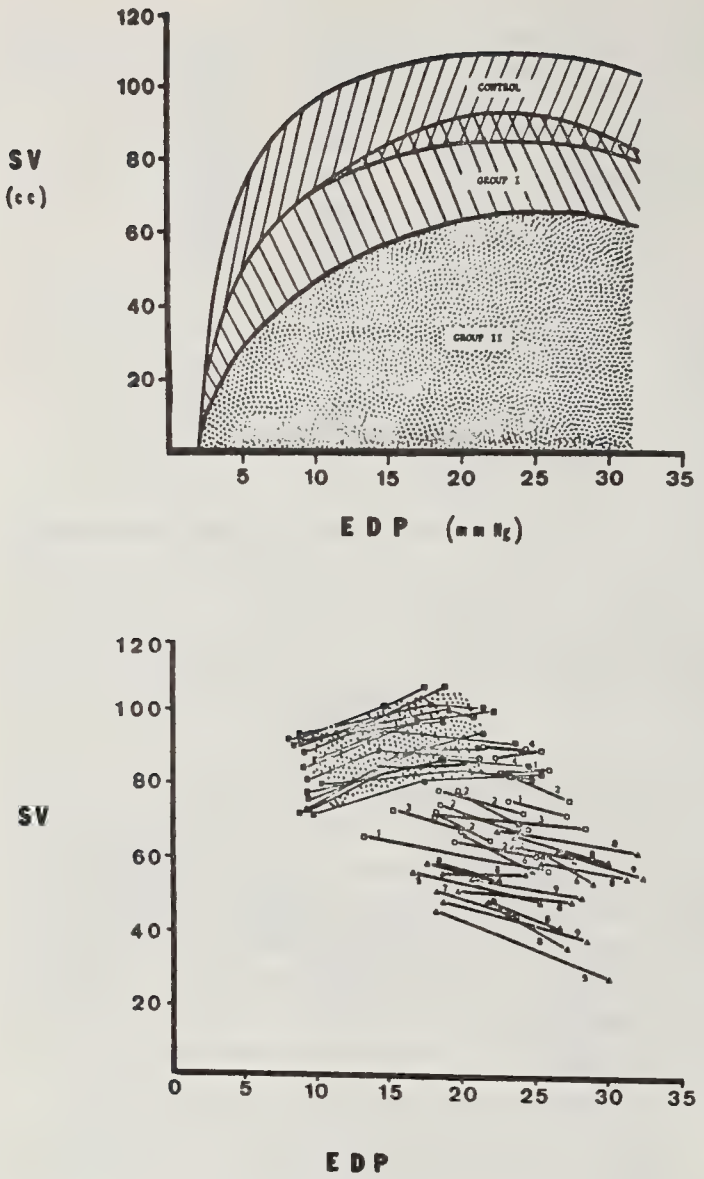


FIGURE 5.—Angiotensin infusion stress curves following subacute microsphere. The control observations for each animal are shown in the speckled area. (Printed with permission of the author from "Left ventricular failure and cardiogenic shock following acute myocardial infarction: A correlation of clinical and experimental observations," *Research Animals in Medicine*, 1972.)

ACKNOWLEDGMENT

We would like to acknowledge Drs. Frank Heck, LaVal Cothran and Mr. John Fuqua for their spirited and creative support during these studies. We also acknowledge the technical assistance of H. Gilmer, C. Poe and J. Wells. We are indebted to Miss Susan Green for her dedicated secretarial assistance. Finally, special commendation is given to the late Dr. Frank W. Hastings, who envisioned and supported the development of the conscious preparation for medical research.

REFERENCES

1. CORSSEN, G., ALLARDE, R., BROSCH, F., and ARBENZ, G. Ketamine as the sole anesthetic in open-heart surgery. Inter Anesth. Res. Soc. 49:1025–1031, 1970.
2. DONAWICK, W. J., and BAUE, A. E. Blood gases, acid-base balance, and alveolar-arterial oxygen gradient in calves. Am. J. Vet. Res. 29:561–567, 1968.
3. BERNHARD, W. F., LAFARGE, C. G., BANKOLE, M., BORNHORST, W., and BUTTON, L. Biventricular bypass: Physiologic studies during induced ventricular failure and fibrillation. J. Thor. and Cardiovas. Surg. 62:859–868, 1971.
4. WEBER, K. T., DENNISON, B. H., FUQUA, J. M., SPEAKER, D. M., and HASTINGS, F. W. Hemodynamic measurements in unanesthetized calves. J. Surg. Res. 11:383–389, 1971.
5. FOLTS, J. D. Preparation of a chronic indwelling catheter using a commercial infant feeding tube. J. Appl. Physio. 30:417–419, 1971.
6. KONIGSBERG, E. ACEMB Conference, November, 1968.
7. KUIDA, H., BROWN, A. M., LANGE, R. L., and HECHT, H. H. Cardiovascular studies in normal calves. Am. J. Physio. 200:247–252, 1961.
8. WEBER, K. T., MALININ, T. I., DENNISON, B. H., FUQUA, J. M., SPEAKER, D. M., and HASTINGS, F. W. Experimental myocardial ischemia and infarction: Production of diffuse myocardial lesions in unanesthetized calves. Am. J. Cardio. 29:793–802, 1972.
9. WEBER, K. T., MALININ, T. I., HECK, F. J., DENNISON, B. H., and HASTINGS, F. W. Experimental production of vascular and microvascular myocardial ischemia: The effects of different rates and degrees of coronary flow reduction in calves, *Reversability of Cellular Injury due to Inadequate Perfusion*. C. C. Thomas, Springfield, Illinois, 226–287, 1972.
10. WEBER, K. T., RATSHIN, R. A., and RACKLEY, D. E. Left ventricular failure and shock following myocardial infarction: A correlation of clinical and experimental observations. *Research Animals in Medicine*, 1972.
11. GOLDFARB, D., CONTI, C. R., BROWN, B. G., and GOTT, V. L. Treatment of severe cardiogenic shock by diastolic augmentation after ligation and division of the left circumflex coronary artery in dogs. J. Thor. and Cardiovas. Surg. 51:783–796.
12. HIATT, N., SHEINKOPF, J. A., and WARNER, N. E. Prolongation of survival after circumflex artery

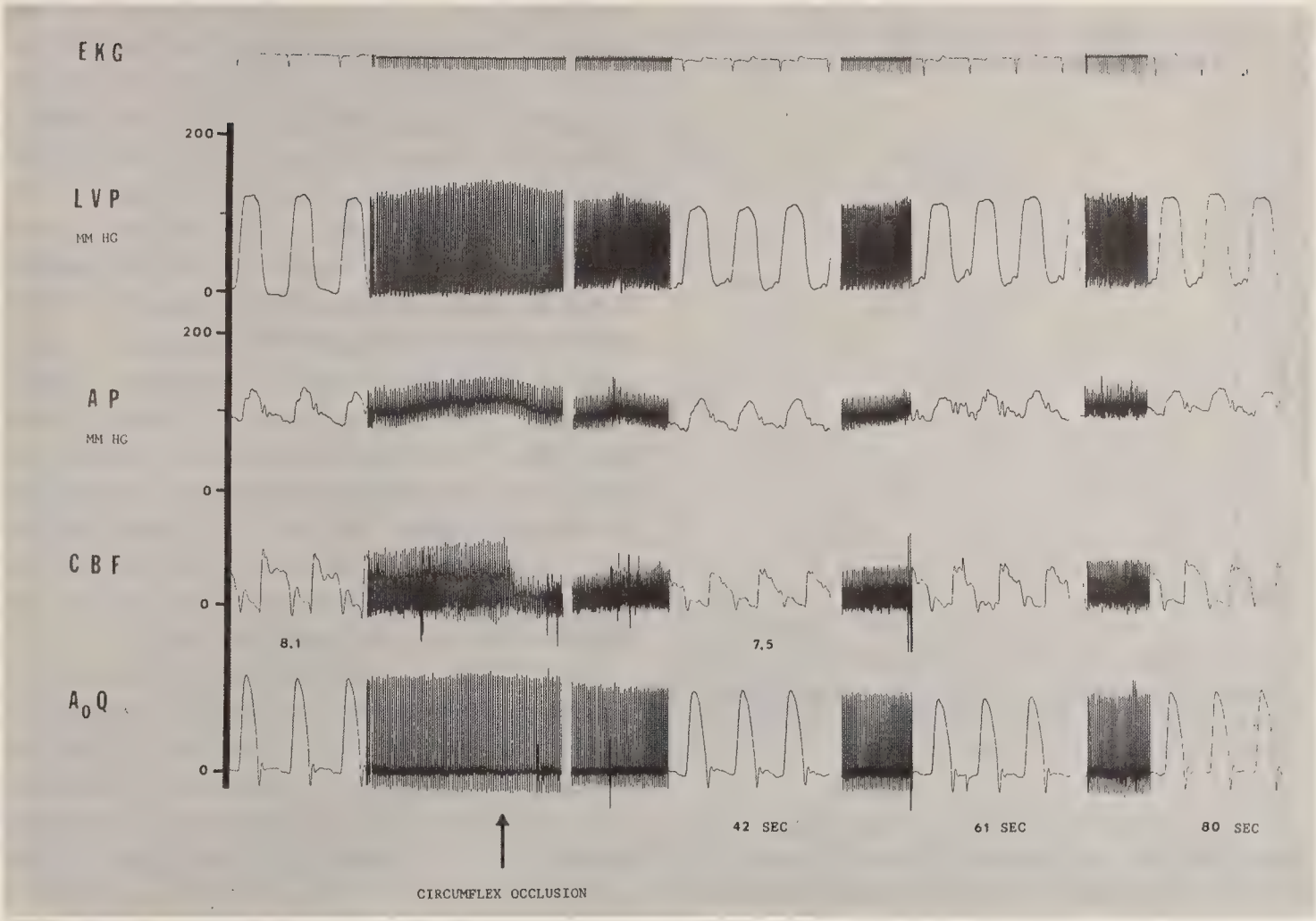


FIGURE 6.—Continuous monitoring during acute circumflex occlusion. Displayed are electrocardiogram (EKG); left ventricular pressure (LVP); arterial pressure (AP); left main coronary flow (CBF); and aortic flow (AoQ).

ligation by treatment with massive doses of insulin. Cardiovas. Res. 5:48–53, 1971.

13. WEBER, K. T., HECK, F. J., DENNISON, B. H., REAN, A. K., and HARMISON, L. T. Intra-aortic balloon pumping: The effects of inflation duration in unanesthetized calves. Supplement II to Circ. 43 and 44:II–238, 1971.

DISCUSSION

H. CLARK, Palo Alto: I was just curious if that was glucagon free insulin that you used.

DR. WEBER: No, sir, it was not. We are currently in the process of evaluating the effect of glucagon-free insulin alone.

DR. CLARK: I would suspect from the rather large doses of insulin that you may have, it's likely you may have a little bit of glucagon effect.

DR. WEBER: Yes, I'm sure we do, and we do see an inotropic effect that is reminiscent of that of glucagon. However, the electrical changes or arrhythmia control that accompany the insulin are not that of glucagon unless you have information to the contrary.

S. CASSIN, University of Florida, Gainesville: Do you have any idea what the largest dose of microspheres is and what this represents in total number of spheres?

DR. WEBER: No, I'm sorry, I don't. I also have no idea as to the quantity of myocardium that is damaged with these doses, but it is a diffuse process involving the entire left ventricular myocardium.

C. G. LA FARGE, Children's Hospital, Boston: Just a brief comment about the balloon

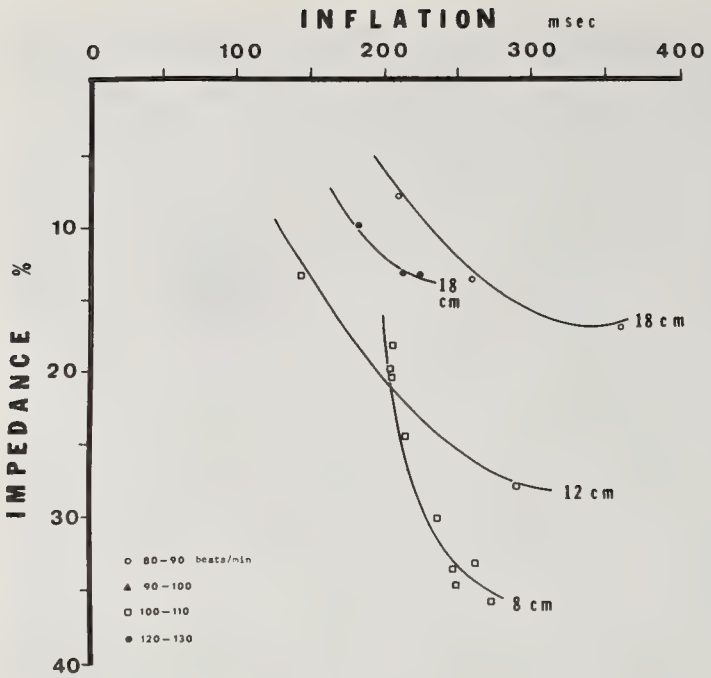


FIGURE 7.—Mean impedance to ejection versus balloon inflation duration for various heart rate ranges. In addition, the influence of balloon position (distance from aortic valve to balloon tip) is given.

cuff occluder lest anybody be frightened away from it by the arrhythmia problem. I know that both you and Jack Norman in Boston have had problems, and I think it stems from the fact that you use complete occlusion rather than incomplete occlusion. In a number of studies that I've done inducing similar failure, often with a greater or higher left ventricular end-diastolic pressure, I've used incomplete occlusion of the main left coronary artery, still allowing a small trickle through. This means that when I release the cuff, there are no arrhythmias, and I have yet to see any ventricular fibrillation.

D. B. JACKSON, Abbott Laboratories, North Chicago: Could we go back and look at your last slide? I am curious about one thing and that is the two curves plotting mean impedence to injection versus the balloon inflation, where the heart rate (the top heart rate and the one next to it) curves seem to me to be very far apart. Could you interpret that for me?

DR. WEBER: I think that the plot (Figure 7) considers not only heart-rate range but also position. One has to realize that in plotting decreased mean impedence the reflection between the two curves would be augmented by the positional effect, if I understand your question correctly.

CHAPTER XIV

THE USE OF ANIMALS IN MEDICAL RESEARCH AND EXPERIMENTATION

THE USE OF IMPLANTABLE TELEMETRY SYSTEMS FOR ANIMAL MONITORING

D. F. Schwindaman, Mark Conner, Charles McPherson, Joseph Pierce, John C. Norman, Julius Cass, Harold R. Parker and Lowell T. Harmison

DR. HARMISON: This session touches upon several aspects of the use of animals, their care and research. We thought it would be appropriate to approach this somewhat delicate situation from the standpoint of understanding more clearly the regulations set forth in the Animal Welfare Act. This will be followed by a discussion of the guidelines for implementation and follow-through, as viewed by the NIH, and a discussion of how the legislation, the Act, and the interpretation of the guidelines fit in with the people who have to use, follow, and execute the information. I think it goes without saying that the scientific community has long recognized the responsibility, both technically and ethically, of the need for guidelines in the care of good animal research. It goes beyond that to include the broader domain of humane care in the total treatment of medicine extending itself to man. So, it is a broad subject. We're dealing only with the first part, that is, with the animal. We're not here to basically discuss the justification or the lack thereof, but to face the problem in a real and direct sense as it presents itself today. From this vantage point, we can see that the scientific community has a major role and a major responsibility.

Without further discussion on my part, let me introduce the panel members who have so graciously consented to participate in this discussion. To my left is Dr. Mark Conner who will be discussing the NIH Guidelines. Dr. Charles McPherson from NIH, the Animal Resources Branch; Dr. D. F. Schwindaman from the U.S. Department of Agriculture; Dr. Joseph E. Pierce from NHLI; Dr. Julius Cass from the Veterans' Administration; Dr. Harold Parker from the University of California at Davis, who really has two views here tonight, one representing his new role as Chairman of the Committee of the American Physiological Society and his own personal views; and Dr. John C. Norman from Harvard to give us a view of how the clinical side of the legislation directly interrelates and comes to bear on the subject of humane care. At this time I'd like to ask Dr. Schwindaman to present a discussion of the Animal Welfare Act.

DR. SCHWINDAMAN: I will start by giving you just a little background on the first law, Public Law 89–544; what led up to the passage of that particular law; what happened in the four years that we were enforcing it; and the amendment that came about with the passage of Public Law 91–579, the Animal Welfare Act of 1970.

Now during the discussion I'm going to be talking about the Old Act, the New Act, and The Act. When I talk about "The Act," I'm taking about the two Acts put together, P.L. 89–544 as amended by P.L. 91–579.

Public Law 89–544, as most of you know, was passed and signed on August 24, 1966. This particular Act came about after several bills had been introduced into Congress in the previous years, but I think primarily it was articles in the public press that really brought about the public reaction to cause its passage. This Act was somewhat limited in its scope. It directed itself toward two aspects; one aspect covered dealers in dogs and cats who sold them for purposes of research or experimentation, and the second covered the research facilities where such dogs and cats were used for experimentation.

Public Law 89–544 was keyed to the use of dogs and cats. There were six species mentioned, but it was oriented to these two species. Now if you used dogs and cats and came under the

* A panel discussion presented at the Conference "Research Animals in Medicine," Washington, D. C., January 29, 1972.

Department of Agriculture's standards and regulations, and you also used guinea pigs, rabbits, hamsters, or non-human primates, then these additional four species had to come under the Department's standards.

Public Law 89–544, as far as many of you were concerned, probably did not directly affect you, simply because it stopped at the research door. That particular Act, the Old Act, did not give the Department of Agriculture any authority to inspect animals that were actually under research as determined by the research facility. This was changed with the signing of the Animal Welfare Act of 1970, the New Act. During the time that we were administering the Old Act, the Department covered approximately 1,500 research facility sites. These were sites where animals were maintained for research purposes. We had licensed a monthly average of around 205 dealers in laboratory dogs and cats. The administration of that Act was done by our field force. Many of you people have had contact with the Department inspectors. Most of my remarks are restricted to the research aspect and not the dealer's because this is primarily the part in which you're interested. The Department's inspectors by policy were veterinarians, so these were veterinarians who were coming to inspect your central animal care facilities. This in essence has been the program for the past four years.

During 1970 there was a bill introduced in Congress to amend Public Law 89–544 and expand its coverage considerably. This bill went into Committee; hearings were held, and the final version that was referred out of the full Committee on Agriculture on the House side was finally signed into law as Public Law 91–579. What did this new law do? Well, there were several things. Number one, I think it took away the emphasis on the animals used for research purposes. It recognized the fact that humane care and handling should apply to animals for whatever purpose they were going to be used. It did this by covering not only animals which were going to be used for research purposes, but for exhibition and for pets.

I'll just mention the exhibition animals briefly. The Act itself specified animals which were going to be used for exhibition in zoos, roadside zoos, carnivals, and circuses. Of course, the thing that it did not recognize at the time was that circuses were primarily made up of individual animal acts, where an owner owns the act and as a result is going to have to be licensed. So there are going to be very few circuses that will be licensed or registered under the New Act.

What about the wholesale pet purposes? Again, it's for animals that are used, with certain exceptions, or sold for wholesale pet purposes. It's somewhat redundant there, "Sold for wholesale pet purposes." There were certain exceptions and exclusions in the definition of dealer and exhibitor that I want to mention because they may or may not apply in the case of a research facility. One exclusion is the retail pet store. The retail pet stores are excluded unless they sell to a dealer or to a research facility. If they do sell to a dealer or a research facility, then they must be licensed. We've found that there are a large number of pure-bred breeders that sell animals for pet purposes, which were going to have to be covered by the wording of the New Act. So in writing the regulation, we have put in the definition of retail pet store animals sold at a retail outlet for retail purposes. In effect this means that these hobby breeders, the pure-bred breeders, will not have to be licensed if they only sell at the retail level. But if they sell at wholesale level, they will have to be licensed. So in procuring some of these pure-bred animals, you will probably be getting them from a licensed source, in any case.

Another provision of the New Act is that it took away the "dependence on commerce" and substituted "affecting commerce." When it did this it actually included any animals in intrastate commerce by the very wording of that particular phrase. So if you as a research facility are obtaining your animals within the state, this means that you will have to be registered even though you do not get them across the state line because they will be burdening or affecting commerce.

Now what is "animal" under the New Act? Animal is going to be the six species mentioned previously and, in addition, any warm-blooded animal that is normally found in the wild,

whether raised in captivity as domesticated. Once an animal is named by the Secretary, it is covered across the board for research purposes, exhibitions, or pets.

We have in the definition some exclusions. These are rats and mice, aquatic animals, birds, and domestic farm animals. So these are not an "animal" under the New Act. It does sound odd that you can have humane requirements for a certain species and not for all of them. But there is a reason for these exclusions, and primarily it's because of a lack of manpower and money. Rats and mice are probably the animals used used in the greatest quantity for research purposes; and in our discussions, we felt that perhaps with these particular species of animals there was not any great inhumane care in handling.

The New Act directed itself more toward the exhibition animals. So this is where we're going to place our primary enforcement responsibility.

Under the New Act there are going to be certain research facilities that can be exempted. These research facilities will be those where no biomedical research is being conducted. They do not use dogs or cats; they use the animals covered in small numbers; and they do not vitiate the purposes of the New Act. To make application for this exemption, each and every one of these will have to be inspected and recommendation made by our administrative people in the field as to whether they shall in fact be exempted. If they are exempted, their names will then be published in the Federal Register.

How is the New Act going to be administered? Well, essentially it's going to be administered the same way as the old one. We're going to have our veterinarians in charge who are primarily responsible for the administration of The Act in their state along with their staff of inspectors who will be making the inspections. Now I mentioned that under Public Law 89–544, the Department's jurisdiction went to the research door. Under the New Act, the authority is there for us to go in and to see that the animals actually under research are held under the humane care and handling standards, and provided the creature comforts, if you will, as long as it does not interfere with the research procedure, protocol or the experimental design. This determination will be made by the attending veterinarian or an animal care committee for the institution and consisting of three members, one of whom shall be a Doctor of Veterinary Medicine. This particular animal care committee is going to have a great deal of responsibility, and I hope they recognize that.

The new law requires that the research facilities submit to the Secretary of Agriculture, beginning February 1, 1973, and each February 1 thereafter, an annual report in which they give the number of animals used for experiments and other items, which include the following.

"(a) The location of the facility or facilities where animals were used in actual research or experimentation; (b) The common names and approximate numbers of animals used; (c) The number of experiments conducted involving necessary pain or distress without the use of appropriate anesthetic, analgesic or tranquilizing drugs."

Now, I know that many people in the scientific community feel that this is going to be a great burden. We hope that it will not be. Here again a great deal of responsibility is being placed upon the attending veterinarian and/or the animal care committee, because the attending veterinarian or the animal care committee will have to certify these experiments that were used without these classes of drugs: the analgesics, anesthetics, and tranquilizers. I think the reason that there is a great deal of responsibility here is that we are also required to submit to Congress a compilation of these experiments in an annual report during March of each year. Now I hope that I can set your minds at rest somewhat, because the annual report is not public information. When it is submitted to the Secretary, we in turn will compile it and submit it to the Congress. By law, the only people who can release this information, and then it will not be identified with any particular research facility, is the President of the Senate or the Speaker of the House. So I do feel that the research facilities have been protected here.

There is only one thing that I would like to mention in addition to the annual report and the use of these classes of drugs, this is the transportation standards under which animals have to be transported. The licensed dealers

have to transport their animals under FDA standards. If you receive animals that are not transported under these standards, we would like for you to let our inspector know. We do not have any jurisdiction over the common carrier. Again, in the new law the common carrier was excluded. But these animals must be transported to your facility under the USDA standards.

Another thing I would like to mention before closing is that we have told our inspectors to identify themselves whenever they are requested to do so. I mention this because I don't feel that you should have any hesitancy in asking our inspectors for identification. They know that they are expected to provide this and they will be glad to do it.

In summary, we had an old law that dealt mostly with dogs and cats. We now have a new law that deals with all warm-blooded animals as designated by the Secretary. As of December 24, 1971, the Secretary has designated dogs, cats, guinea pigs, rabbits, hamsters, non-human primates, and all warm-blooded animals that are normally found in the wild. The authority for additional species of course is in the New Act. We don't feel that these will all be so designated in one year. We feel that because of lack of funds and personnel, we'll have to phase into this, but nevertheless I think that, as research facilities, you are going to have to expect that sooner or later all warm-blooded animals will come under Public Law 91–579.

DR. HARMISON: Thank you very much, Dr. Schwindaman. I would like to reiterate that point. I think that many of the viewpoints and information that will be presented by the panel will go a long way in providing much needed information for the researchers. The next formal presentation will be by Dr. Mark Conner, who will discuss the NIH Animal Research Guidelines.

DR. CONNER: I think my remarks should be prefaced by a brief administrative identification because I'll be talking primarily about an NIH policy, yet I'll be referring to the Department of Health, Education, and Welfare and the Public Health Service and that might be a little confusing. I am from the Division of Research Grants at NIH, which is I think a name familiar to many of you because that's where your research grant proposals go, even though in many cases they are not directed to an NIH program. We still service a number of units of the Public Health Service, so that's where they are received, processed, and given their initial scientific review at the Division of Research Grants. That still doesn't explain why I will say later on that we are administering a Public Health Service policy or a DHEW policy. The fact is that this sort of thing "just grew like Topsy" primarily because NIH was the chief focus within the Department for the performance of and support of studies in the biomedical discipline, so that's where the administrative and scientific expertise was concentrated. At the moment I am in a section of the Division of Research Grants called the Institution Relations Section which has two chief responsibilities—the administration of this animal welfare policy that we're going to be talking about and the administration of the human subjects policy of which I am sure most of you are aware. So, this is a case of an individual entity at NIH acting in a service capacity for the administration and development of policies for the Public Health Service and the entire Department.

One of the things that we have been asked to emphasize is why should we have regulations or policy requirements at all on this business of taking care of animals. It seems that they have probably been taken care of fairly well for many years. Why do we have to be restricted by certain procedural requirements? Well, there are a number of reasons for this.

Starting back in history, I'm sure that most of you are familiar with the Policy Statement on Grants for Research Projects and a similar one for training grants. The Policy Statement was dated 1967, and at that time, it had a statement about animal procurement and care. It emphasized, as you might expect, the law administered by the USDA and went into some length to describe it. Then it simply said that it was the responsibility of each person assigned to or appointed to a PHS project to exercise every precaution to insure proper care and humane treatment of research animals. Well, this is directed toward the investigator, rather than the institution in which he's working. That

is really contrary to a basic philosophy of our policy for research grants and training grants because the money goes to the institution. The institution is considered the grantee, not the man who's directed the project. So, this regulation, by emphasizing the investigator's responsibility, really was contrary to our long-standing philosophy and did not put the responsibility where we thought that it should be—on the corporate entity that received and managed the funds that the man used to carry out his project. This was one reason for saying something more than what we had been saying for a number of years (to put the responsibility where the money was) because the only control that we really have over how our grants, contracts, and awards are carried out is the disbursement of money. In other words, the only way that we could stop something that was not right would be to withhold funds from it. This is not an insignificant method of control, but this really didn't speak to that point. That was one reason why many people thought it ought to be changed.

Another fact is that over the years our study sections and councils have seen a number of projects submitted to NIH and the other Public Health Service agencies that did suggest that the investigator proposed to do something that really wasn't very nice with animals. I guess this averages about four or five proposals a year, not many, but enough to develop a certain amount of concern in many peoples' minds as to whether some of the institutions that were receiving our grant money really were aware of what some of their investigators wanted to do with animals because some of them were rather horrendous. We have a good screening mechanism for this sort of thing called a Study Section. As you know, these are panels of outside consultants from your own universities that review our grant proposals and take note not only their scientific merit but of these kinds of things. Over the years, a handful of these projects that were quite off-base had been called to our attention. Certainly the development of the laws administered by USDA has had its impact on our thinking, both the original Act and the one that was amended as of Christmas Eve one year ago.

Another thing about these so-called "problem projects" that we see from time to time should be emphasized. I think without exception they were recommended for disapproval and never funded. This simply is a reflection of the fact that if an individual is willing to practice poor husbandry he probably is a rather poor scientist, and the way animals are treated, held, maintained, and cared for would certainly affect the expected outcome of the research that's going to be performed on them. So if it's part of our mission to improve the quality of biomedical research, we felt that we ought to encourage institutions and their employees to do the best they could about the way they deal with animals, both in the holding facility and in the laboratory itself.

A third thing that certainly must have influenced NIH to develop this administrative policy is the longer experience with the more intensive requirements on human subjects, a thing that some people consider to be even more important than animal welfare. This developed originally in 1966 and has become more and more precise and detailed in its requirements. Experience with it has shown that its existence has resulted in our receiving fewer and fewer proposals that would be considered problems so far as what the man intended to do with human beings. I'm sure that we have hoped that the same effect will take place on laboratory animal research.

Last but not least, we certainly are aware, as everybody is, of the increased public interest in all aspects of scientific ethics. I think that every scientist, particularly those who are dealing with living systems, are under closer scrutiny than they ever have been before, so that we are not really quite as surrounded by ivory towers as we once were. I don't think anybody will consider that an unhealthy situation, but it's one that I think we ought to consider.

So, these are some of the reasons that I would give as why NIH should have administrative requirements tied to its grants and contracts mechanism. That is a point that should be emphasized in comparing it with what USDA administers. USDA, Dr. Schwindaman's office, administers a law. We are talking about an ad-

ministrative policy. It is not law, but again since it's tied to grant and contract funds I'm sure that it's almost as effective as if it were a law.

The present NIH policy we're talking about tonight was published round the middle of June of last year. I'm sure that most of you have seen it or at least somebody in your institution has, and most of them have replied to us. In essence, it requires your institution, if you use significant numbers of the six species the USDA law speaks of, to select one or another of two different courses to assure NIH that you're prepared to use proper standards and methods in dealing with laboratory animals. You can either seek and get accreditation by a national accrediting body, of which there is only one that we know and that's the American Association for Accreditation of Laboratory Animal Care, or you can set up a committee in your own institution composed of at least three members, at least one of whom has a Doctor of Veterinary Medicine degree, to oversee your activities with these six species that are covered. This would really involve all aspects of what is done with these animals, so that it's quite possible that these institutional committees could get into the business of knowing something about the man's research protocol and calling it to question if they feel that it is out of line with recognized humane requirements or expectations.

We have had many inquiries from institutions "Where we are, we have to license ourselves with the USDA. Does this not suffice?" The answer is no, it does not suffice. This is not a licensing requirement. It is a requirement for accreditation and self-examinations by the institution. Other institutions have asked us, "Well, we're in a state that has a state law that requires us to be inspected periodically and certified by, say, the Department of Health of the state. Does this not suffice?" Again, the answer is no. Our requirement is quite different from this. It does not pertain to inspection and licensing or certification. It expects either accreditation by a nationally recognized body or ongoing overview by a faculty or institutional committee, including a Doctor of Veterinary Medicine. Frankly, in some cases when we have dealt with institutions in an intellectually and geographically isolated area, we have had to think long and hard about the possibility of waiving the requirement for a Doctor of Veterinary Medicine. Not in many, but in some cases, I'm sure it's been possible for them to convince us that a Ph.D. in physiology or something like that with eight years of experience in using animals, in that particular place, might be preferable to the DVM down the way who's in farm animal practice, whom they could get in if they wanted, but whom they'd have to pay a fee, and where does the money come. So, we have tried to be reasonable. This is something that most large institutions have been able to comply with without any trouble.

To date, we have heard from 1,700 institutions. There are a few notable exceptions. We pointed out that it was desirable to have heard from you before January 1, 1972, in some fashion, either "no you don't use these animals," or "yes" you do; you've got AAALAC accreditation or you've got a faculty committee. We are in the process now of preparing a list of institutions that we have had satisfactory responses from and this list will be distributed to all NIH programs, and they will be required to look at this list before they award a grant concerning the use of laboratory animals. The policy doesn't go beyond that. It doesn't tell them that they must not award that grant simply because the institution is not listed, but it would certainly have a bearing on a project on which a Study Section or Council raised any questions of ethics or any questions about how these animals are going to be dealt with. I think it would be to the benefit of all institutions if you would find out whether your institution has responded to this inquiry. We've put out at least two communications on it, including the NIH Guide issuance.

I think a word about the possible future is appropriate, too, because this will certainly have a bearing on your response to NIH. At the moment our office is busily working on a redraft of this policy that will expand it and make it applicable to all agencies in DHEW. It will have the chief effect of bringing in certain other DHEW entities like the National Institutes of Mental Health and the Food and Drug Administration that are not covered now, where there is a significant amount of animal

work supported by their grant and contract mechanisms. Beyond that, the line, word and verse of the policy will be strengthened to a degree.

I think quite definitely one of the things that will change is that it will apply to all warm-blooded animals and then let you define what is a warm-blooded animal, but the exclusion of rats and mice I think is something that's going to rapidly disappear. We will have to wait and see exactly what form it takes because it will have to be studied by the Departmental task force committee, but this is on the way.

In conclusion, remember that for your NIH grants and contracts, there is something now that requires your institution to do something; and in the very near future, it will apply to grants, contracts, and awards from other DHEW entities.

DR. HARMISON: Next I would like Dr. Charles McPherson to give us a view of how he sees the policy and the guidelines influencing animal resources.

DR. CHARLES MCPHERSON: Thank you. You have heard this evening of legal requirements for the care of laboratory animals and also about the NIH policy on the care and treatment of laboratory animals. However, for meaningful compliance with both the law and the policy a number of institutions are going to have to spend some money to improve their animal programs, improve their caging, and improve their facilities.

Congress recognized this when they passed the Animal Welfare Act, and they recognized it again last year when they made the appropriations for fiscal year 1972 since they added $1.5 million to the budget of the Animal Resources Branch of NIH. We have specifically ear-marked this money for the purpose of helping institutions comply with the NIH policy and with the Animal Welfare Act. These monies will be distributed by a competitive grant mechanism. We of course have had an Animal Resource Grant Program for a number of years, and we support a variety of activities which are designed to help institutions and groups of investigators improve their animal resources so they can conduct research more effectively.

Now this is added to that program. I said that they would be competitive grants. The competition will be based both on needs and the overall quality of proposals. The need will be judged in relation to the medical research requiring laboratory animals in the institutions and the actual requirement for assistance. We obviously are not going to spend money to help institutions that don't really need it, if their facilities and programs are now in good condition. The quality of the program will be judged on the basis of the overall plan, and the items that go into this are the administrative arrangements for the Animal Resource Program.

The Animal Resource Program has to be responsive to the requirements of the investigator because that is its purpose, but it also has to have a firm administrative base. It has to have institutional support and it has to be organized in a manner so that any improvements that are made will be lasting; it is not a matter of going in and buying new cages and papering over any deficiencies. What is really required are the changes in the organization and changes in the commitment of the institution to maintaining a good animal resource.

Of course, the ability of the program to markedly upgrade the quality of care will be considered. Are the changes they are making really going to be significant? For a very mundane example, are the cages they're proposing to buy really what is required to uplift the quality of care? Are they appropriate for the species and the research? Perhaps most importantly, is the staff of the animal resource program that the institution proposes competent to carry out such an improvement program? These are just a few of the things that are judged when we look at the overall quality of the program in determining the competition for these grants. Thank you.

DR. HARMISON: Next, I would like Dr. Joseph E. Pierce from NIH to discuss the impact on his laboratory and give us his views concerning the Animal Welfare Act and Guidelines.

DR. PIERCE: My experience as a veterinarian in the laboratory has convinced me that there are many inadequacies in the use of animals in the laboratory and the law presented here

tonight is very necessary to correct some of these inadequacies.

I think it is appropriate that we include as part of our presentation an examination of certain practices in the laboratory involving research animals. The purpose is to examine whether we are providing the necessary laboratory organization not only to accomplish the necessary goals of scientific achievement, but the best use of laboratory animals in these goals. I am speaking in relation to experimental protocols involving animals in research. At this time regulations of the animal welfare law do not affect the experimental protocol. The law specifically exempts experimental design or activity from regulation. In this country, the investigator has the privilege of using, in a humane way, laboratory animals at his discretion. I feel that there should not be limitations of this privilege except when an investigator fails to recognize and correct inadequate techniques that, while humane, cause unnecessary use and loss of animals. When we allow conditions, practices, poor techniques to exist that are not advantageous for the expediency of biomedical research, we have to respond with what it takes to correct our deficiency.

Many of us feared the enactment of the law last year that regulates the care of animals involved in research, not so much because of inadequate facilities that we could replace in sufficient time, but probably because of the exposure of inadequate techniques as well as technical and professional staff maintaining animals in our laboratory. The law now requires that a yearly report be submitted listing intended experimentation that will involve animals exposed to "pain" without benefit of analgesics, tranquilizers, or anesthetics, along with a verification that all other animals exposed to pain are treated with proper drugs and dosages of these drugs.

Animal welfare legislation has the support of "we the people" primarily because unnecessary abuse of animals in the laboratory as well as other locations has been recognized. Other deficiencies exist! What are we going to do about them?

Cruelty to laboratory animals can be represented by the misuse of animals in experimentation. Here are some examples that now exist: The use of unhealthy animals in experiments; failure to select the proper species; failure to have a protocol adequate to cover the extent of an animal's involvement in an experiment; failure to have qualified technicians and professional assistance; the production of misleading information from misuse; failing to accomplish experiments with the minimum number of animals required; inadequate post-operative treatment; inaccurate clinical evaluation of the recovered animal at a particular stage of experimentation and so on.

Our concern with these examples should begin before experimentation is underway. Concern should range from not only the justification for using animals for an experiment, but the selection of the best suited species for the area of research to be attempted. We must have a holding facility that is sufficient for holding the animal species whether it is overnight or for an indefinite period of time. There must be available an animal care staff capable of properly maintaining the species. It is necessary that we start with a normal animal—one that is of good health and shows no signs of infection, malnutrition, or physical deformity as determined by a qualified professional staff member.

We must have adequate laboratory preparation before scheduling the procedure. This includes equipment, drugs, experienced technicians, as well as an investigative staff capable of performing the desired technique and properly maintaining the animal. If post-operative survival is part of the protocol, necessary preparations for post-operative treatment and recovery have to be arranged. Qualified personnel must be available to treat the animal symptomatically, not only during surgical recovery, but during the duration of its maintenance in the laboratory. Consideration has to be given for extra precautions necessary for the management of the implantation of artificial devices as compared to simple thoracotomies. The post-operative technique should include a daily recording of all recovery events including body temperature, blood chemistries, appetite, as well as necessary treatments. This is necessary for evaluating recovery events of animals before physiological data is to be subsequently

recorded. Failure to follow the above criteria will usually lead to the unnecessary loss of experimental preparations as well as the use of sick animals mistakenly assumed to be healthy.

How many of us are concerned enough to consider reasonable clinical evaluation of an animal before using it for study? How many of us have studied animals that were several days post-operative without a body temperature recording or at least a stethoscopic examination? How many of us have used the presence of a heart beat or the animal's ability to wag its tail as an indicator of recovery. How many of us have been concerned about the hemogram of a dog, sheep or calf each time that one has been used in an experiment? How many animals are hyperthermic from infection, or dehydrated from diarrhea, or anemic from parasitism while involved in study? Would it not be appropriate to include such observations when results are considered?

What can we do to upgrade the use of animals without affecting the laboratory environment as we know it today or without the necessity of further enforcement of regulations for animal welfare? First, let us examine our present laboratory organization and determine whether we have the practice of veterinary medicine at a level to provide maximum treatment and support of all animals involved in our research program. The availability of adequate veterinary support does not mean that all problems will be solved, especially if the investigator fails to recognize it as a necessary assist. Also, the veterinarian has to become involved in an area of animal research that possibly does not stimulate his interest. The veterinarian must assist in all phases of animal experimentation whenever animal welfare is threatened. He must become much more involved than monitoring anesthesia if other deficiencies exist.

Every research institution that includes laboratory animal surgery as part of its program should continue developing means to review all animal experimentation within its program. The institutional committee recommended by law should have jurisdiction over all laboratory protocols involving animals. This not only limits questionable laboratory practices but will stimulate the development of improved experimental techniques within the organization.

Also, we should look toward the editorial committees of scientific journals for help in approving and enforcing criteria on monitoring of certain laboratory animal preparations used in research that result in the publication of physiological data. Such criteria need not limit the use of laboratory animals but would lead toward the limitation of unacceptable practices which now allow the publication of inaccurate data. These criteria would aid in the definition of normal or otherwise identified animals used in certain preparations as identified by the required standards.

Have we all considered introductory courses on laboratory animal medicine and surgery techniques as a requirement for medical and graduate students? All of those people who will be using animals at some future date for research should have this experience. There are several active programs in progress today. We do not want to limit such courses to animal care personnel. The investigator should have some insight into the requirements of laboratory animals before he becomes involved in experimental protocols. We should stress that all potential animal investigators be required to have certain basic courses in laboratory animal medicine and surgery.

There are other inadequacies that we could continue to mention, but why list them here? Where will we get the money? Where will we find the time to correct the deficiencies that we are all concerned about? We are all aware of the continuous developments surrounding the laboratory. We must realize that these developments are not limited to the updating of equipment or changes in personnel. They include the education, acceptance, and practice of constantly improving methods of animal experimentation and maintenance.

In conclusion, the acceptance of and the advancement of veterinary medicine in the laboratory is not the whole answer. It has to be a total collaborative effort on the part of the investigator, veterinarian, technician, and an involved administration. The development of informative techniques to reach the young investigator as well as the now more active investigator must be pursued as well as the

acceptance by the scientific community of the development of certain criteria to eliminate poor technique in the use of animals in experimentation.

DR. HARMISON: I think this underscores very clearly something which we all recognize, that animals are something more than a bottle of chemicals to be used and resupplied at a regular interval. At this point I think it's appropriate to now turn and look at this subject from a slightly different viewpoint, the clinician's direct viewpoint. I would like Dr. John C. Norman from Boston to give us his views.

DR. JOHN C. NORMAN: I will begin by briefly giving you a bit of background. We have a laboratory in the hospital, at the large trade school in the northeast, in Boston, and it has a relatively large faculty. I won't go into the specific university, but I think its bureaucracy exceeds that of the megalopolis on the Potomac that's called NIH. It's a bewildering place to work but suffice it to say that there are about 2,000 people on the Harvard faculty, about 18 teaching hospitals, about 600 medical students in varying states of disarray come through it year after year.

I think we've been made aware of the old and new laws, P.L. 89–544 and P.L. 91–579. I would hope that our compliance has been filed. I think we're all constrained by the quandary as to what to do about situations like this and since at Harvard all of the our lectures are still in Latin, I brought along three quotations.

The first is "Avtor alia est," and essentially that means "the law is the law." I was reminded of another which my Latin teacher taught me many years ago down in West Virginia. "Pars cum partis," that is to say that "birds of a feather do flock together," and we're here concerned about the same sorts of problems.

And finally, the terms of the problems that are involved, the last (I'm sure the fellows from Houston will know this): "Illegitime non carborundum." "We will have to persist."

We don't have the benefit of a veterinarian in our laboratory. We run a very small unit, but I think we've done five or six thousand experiments in it over the past seven or eight years. I'm not quite sure where I should begin in terms of how we as clinicians feel. I'm in cardiovascular and thoracic surgery. So we do surgery on patients in the morning, and/or afternoon, and we work in the laboratory earlier in the morning or later in the afternoon and at night.

Now one thing I would like to leave with you is that we apply to our experimental animals the same criteria that we apply to patients. We treat our animals with a certain respect and we become quite close to them. I'd like to give you three examples in terms of animals that have stayed with us in the laboratory over two- or three-year periods. Dr. W. Jean Dodds knows about our hemophilic animals that we've flown in from Oklahoma from her laboratory, and from Ontario, in terms of splitting transplantation for hemophilia. We fell in love with them immediately. Two of them lived in my office and made a shambles out of it over a two-year period, primarily because we were concerned about their welfare.

Dr. Harmison and, before him, Dr. Hastings were aware of the animals we had in our laboratory over a three-year period in terms of nuclear power sources for artificial internal organs. They were extremely valuable. One was a little difficult to deal with. His name was Wolf. But we became attached to these animals and when we did sacrifice them, we did it with grace and style and had Dr. Harmison in Boston to make sure that it went along well.

I think that in terms of concern, in terms of academic medicine and the formulation of public policy, it behooves all of us to be aware of problems. A specific example of this was a phone call I got from a third-year medical student's wife about three weeks ago. We run an experimental surgery course for our third-year medical students, and you know the current posture of medical students, the ones who were around university halls in 1968 and 1969. They don't mind making us aware of problems. It was about 11:00 at night and I was in the laboratory per usual. We were going over contracts and grants and study sections, and getting ready to go on site visits, and her husband had been with us in experimental surgery that afternoon. We run a short course, it lasts seven weeks. They do minor and major procedures on experimental animals, and those animals are subsequently used in some of our experiments.

To make a long story short, his wife, who was not a medical student, wanted to know whether I was taking good care of the dog her husband had operated on that evening, whether that dog was getting pain medicine, and whether I would make sure the endotracheal tube came out. Now, I am a surgeon and there is such a thing as a surgical personality, but after the first five minutes of the conversation which lasted about half an hour, I came to realize that she really had a certain point because we don't give some of our experimental animals pain medicine following some of the procedures. But we do give the same patients, patients in the Intensive Care Unit, on the accident floor, on the wards, medications around the clock. I wanted to share that one thought with you in terms of what our responsibilities should be, can be, and probably will be as we come to realize that each soul is precious.

I've got a little dog at home by the name of Sabrina. I usually never get home at night until 10:00 or 11:00. We're out in Concord which is about twenty-five miles outside of Boston, when I get there my wife is asleep, and the daughters are asleep of course, but Sabrina always meets me. I always think of her when I'm working on my animals who come through the laboratory. We also have a Great Dane by the name of Hither that just came up from Stone Mountain, Georgia, and they're part of the family.

In terms of our livelihood, research is something that drives us in terms of looking for innovative thoughts, new insights, and new approaches. Obviously we want to do the proper thing, and I think that our concern should be much more with our animals. I'm reminded of one occasion in our laboratory over the past seven years in which I felt an animal was not treated well, and we removed the personnel involved with that treatment. But by and large, I think we can speak for our laboratory in saying that our animals are treated with dignity.

We can come to the wrong conclusion with the wrong model. We've been going over electron microscopy with Carl Teplitz for a presentation wherein we might have seen pulmonary fibrosis and attributed interstitial edema to that fibrosis by electron microscopy. It's extremely important to look at the model. It is extremely important to be on solid ground. Obviously if our mission is to disseminate information to the academic community and, in the long run, help patients, we must be on a firm base with our animal models.

In terms of the worth of all this, cardiovascular surgery, my particular specialty, has burgeoned over the past ten years. Perhaps all of you in this room may well die of myocardial infarction. But 20,000 patients last year had aortocoronary grafts, a procedure which didn't exist three years ago and was worked out in the laboratory. In terms of mitral valve replacement, there are now 50,000 patients in this country who have mitral valves of prosthetic types replaced in the last five years. That procedure was presented in surgical form in 1961.

In regard to congenital heart disease, in 1938 Dr. Robert Gross at Children's Hospital in Boston ligated the first patent ductus, but he learned how to do it the year before on an animal that happened to have a patent ductus arteriostenosis in the laboratory. When Harvey Cushing at the time of World War I ran up against a very tough board of overseers and trustees of the Peter Bent Brigham Hospital, but convinced them that the proper study of man was not only mankind but the experimental animal and demanded that there should be a laboratory, he brought to Boston the expertise which was developing at Johns Hopkins at that time.

Finally, our involvement is that of a responsibility. We have the responsibility to formulate policy with NIH; our medical school work is vitally dependent upon this sort of situation, but certainly there are flaws in our current practices. I'm certain in ten years what we're doing now will be considered antediluvian.

In closing, another quotation, for Dr. LaFarge who likes poetry. You must have read Robert Burns' "Ode to a Field Mouse," so as we approach our animals we might keep this in mind, "Wee, sleekit, cow'rin, tim'rous beastie,/ O what a panic's in they breastie!" You must remember the rest of it. Finally, from Burns again, "O wad som Pow'r the giftie gie us/To see oursels as others (our animals) see us!"

DR. HARMISON: We do recognize that animal

research is deeply rooted in almost every aspect of clinical medicine. Next I would like to have Dr. Julius Cass give us the views of the Veterans' Administration in this important area.

DR. JULIUS CASS: I too come from an affected agency. I bring no funds or suggest how you might get them. The VA, like all other governmental agencies, is affected by this law in that they themselves must do their own policing and be responsible for following it. We meet the NIH policy, although there is a long-standing ruling that one governmental agency does not have any responsibility for another, in that many of our investigators in VA are NIH grantees who are associated with medical schools and other research institutions. In that way, Dr. Conner, the policy of NIH reaches into the Veterans' Administration.

I would like to present a few thoughts on the fact that the animal research has been badly split down the middle. It has two components now that don't even speak to one another and are budgeted in two entirely different ways, and these methods are such that many times one or the other of these aspects can fall between the cracks because one doesn't know what the other is doing.

Let me show a case in point. Research is defined as an investigator, his concept as designed in his experiment, the procedures that he selects, and the subject which he uses to study. I don't think I'd get any arguments on that definition of research. The concept, the design of the study, the procedures selected, and the subject studied. There are only different media that we can do our studies in: human subjects, animal subjects, the lower animals, biological materials from either of these, and one which we don't relate to a great deal, but does occur, plant subjects. These are the subjects of our study.

In the VA we have clinical research and facilities. We have laboratory research and facilities where the biological materials and inanimate materials are studied, and we have animal research and facilities. Of these three—clinical, where human subjects are used, the animal subjects in animal research and facilities, and the laboratory research and facilities, where biological materials are studied, only one is split up into two units, animal research and facilities. You'll find the animal subjects of research as a supply object. We are budgeted differently, from different sources, administratively, or through a university in a fund that many times has nothing to do with research as we know it. Animal research facilities have no reason to exist in an institution except as generated by the research program. Otherwise, it's an absolute luxury with no purpose.

The laws that we have had enacted address themselves to the humane aspects of animal handling. The word "care" as originally developed was totally associated with the word "humane." The word "humane" has been dropped, the word "care" exists and we've adopted it. Yet involved people that developed this had only one thing in mind, and that is the *humane* care of laboratory animals. The concept does not address itself to scientific validity of handling, manipulating, monitoring, and measuring which includes humane handling and care.

The Guidelines that the National Research Council developed through the Institute of Laboratory Animal Resources Committee and which was published by the National Institutes of Health, Public Health Service go beyond humane care. They include some of the aspects of scientific validity, and we should recognize that. In complying with the USDA law (and I must congratulate the efforts of the USDA under very trying conditions), they have come in to the VA at our request in one instance and have been contacted in many instances at the request of our own investigators in the field to walk a path that is most difficult, not only from the emotional standpoint, but from trying to find fact and put some basis under these humane laws.

But they say, "We stop where Congress has indicated by intent and content of the law that we may regulate," and they do not go beyond this point, even though as veterinary medical people learning more about the field of laboratory animal medicine, science and technology, they recognize that there are areas apart from the scientifically valid part of our work where they could contribute, but they don't step beyond the law. That is why they will not relate

to all of the things that occur, I feel sure, in the policy of the NIH.

I would like to tell you some of the playback that has occurred over recent years, where the law has come into effect by speaking to the use of terms when we speak about these various fields. When I have spoken of these matters with many of you, the statement generally comes, "Well, that's what we intend but we may be using the wrong word." I would like to suggest that in using the word "care" we are not addressing ourselves to the total setting in which we are working; the setting being the animal, its definition, its biological characteristics, its state at any point in time, and its surroundings, both before and throughout studies we carry on. This setting is no different from a hospital setting, where the doctor comes to treat his patient; it doesn't in any way usurp his prerogative as a professional. The dietitian knows her job, the housekeeper knows her job, the clinical laboratory knows its job, the nurse knows her job, and if there need to be changes in this setting, the physician requires it, writes it down and it's done. But the setting is primary. We're dealing with the same kind of thing with the animal in its surroundings.

The animal subject (let's quit calling it a model except as we mean a model) is a subject from which we derive all of our initial data, all of our initial observations, all of our initial biological materials. And yet what investigator among us would be willing to accept funds to do a research program that did not have instrumentation that was sensitive and reliable enough to yield the answers to our questions or the analytical method that was sensitive enough. We would refuse to do that study. But we don't refuse with a fully compromised animal subject from which we derive the data initially. I don't care how good the instrument is that follows that initial piece of material you obtain, you will not correct the variability with good instrumentation.

Using the term "animal care committee" weds us to the fact that this is a humane aspect of the work. In the case of human studies, they call it the "human studies committee" which is great because it reviews the protocol, it addresses itself to the totality of the setting, and the procedures, and their humaneness in the case of man. The parallel to this would be the "animal *studies* committee," not the "animal care committee," and, as a matter of fact, Dr. Conner addressed himself to the fact that it wasn't only the humane aspects with which this committee would be concerned. It could assist in protocol review on animal subjects being used. Using the word "holding facility," for example, suggests there are no observations made there, no monitoring done there, and it could just as well be a bunch of equipment put out into the back storage room.

These are words that have made a second and third priority of the animal subject and its surroundings from which we derive our initial data. I sincerely hope that we don't simply pass that off by saying, "Oh those are just words." Congressmen do not have the background. Personnel people who are classifiers do not have the background and look upon people who feed and water animals not as people who affect these animals, but as janitor types and they classify them accordingly. When we want to upgrade the quality of the animal setting we use, we are faced with the statement that first the man doing the research must receive his money to support his research and then we'll look at the facilities from an entirely different standpoint. It doesn't make sense and I hope we join these two once again as with clinical research and facilities for human subjects, and laboratory research and facilities for biological materials.

DR. HARMISON: I would like to ask Dr. Harold Parker to give us the views of the American Physiological Society, the committee which he's chairing, and some of his own personal views on this subject.

DR. PARKER: It is a pleasure and honor to be asked to participate in this panel discussion as a veterinarian and physiologist.

Since I am wearing two hats this evening I will first speak as a veterinarian-investigator. Requirements of the new law—The Animals Welfare Act of 1970—and the NIH Guidelines pose no particular problems because they spell out objectives which veterinarians believe in. When we became veterinarians we accepted responsibility to work toward improving health

of animals by tending to their needs. In some ways the law and NIH guidelines will help us apply the principles to which veterinary medicine as a profession is dedicated.

I have discussed the impact of the law with a number of my colleagues. Some are in private practice, some involved in veterinary medical education and others hold positions of responsibility managing research animal facilities. In all cases I have been told that they believe the law is good and will have beneficial effects regarding handling of research animals.

I will now switch to my hat as a physiologist. Again I reiterate my statement that there is nothing in the law which will upset physiologists because as a profession we have always adhered to a policy of humane treatment for animals.

We also believe that animal research is essential if we are ever to understand the many mysteries of the animal body. Progress in medicine demands that this kind of research be done. As Doctor Jack Norman so eloquently stated a few minutes ago, there are many people alive today who could not have had their illnesses cured or controlled had it not been for medical research performed on animals. In the future animals will continue to serve humanity by providing biological information which will help us learn how to control crippling and killing diseases.

With due respect to the Department of Agriculture with its new law and NIH with its Guidelines for the Use of Experimental Animals I must state that the American Physiological Society has had requirements for the humane use of experimental animals which preceded the new government regulations. For many years The Society has had its own Guiding Principles for Care and Use of Animals. The principles are published in our journals and are enforced through editorial policy. Every referee editor when evaluating a manuscript must certify that in his opinion the principles had been adhere to when the experiments were conducted.

In order to increase our capability to assure that the principles will be followed the Council of APS has enlarged its Committee on Animal Care and Experimentation and I have the honor of being chairman of the large committee. I am now speaking to you in that capacity. Two other members of the committee are in the audience tonight because of their interest in the new regulations. One of the responsibilities of this committee will be to help physiologists learn what they must do to conform to the regulations governing animal experimentation.

In my opinion the research community has a vested interest in the regulations and will ultimately benefit from them. As investigators we must help each other understand the meaning of the laws and how to abide by their requirements. One way would be for those societies which do not already have editorial policies designed to encourage adherence to the law to establish such editorial policies. I have here one of the forms which is used by APS when a manuscript is evaluated. Members of the audience are welcome to examine it to determine whether it might be helpful in their own situation.

Lastly, Mr. Chairman, it appears to me that within the new law it is implied that new standards will be set, and if they are set it may make compliance with the law more difficult unless they are based on sound scientific principles. For an example I will use requirements regarding dog runs. I have heard rumors that there may be stricter regulations for provision of dog runs. Before a standard like this is written into the law I believe that NIH should accept responsibility to see that definitive research is done to fully establish the need for such a standard, and that the standards be based on firm scientific evidence rather than empirical information as many now are. I am not condemning the use of empirical data because I realize it is the best information currently available to us. I believe we must have better information.

Requiring runs would obviously be for the purpose of permitting dogs an opportunity to exercise, and no physiologist would argue that exercise is not important to help keep animals healthy. However, as most of us who have worked with dogs know, simply providing them a run will not guarantee they will use it. Many dogs will merely sit in a corner and in reality will be no better off than if they remained in their cages. If evercise is the objective there

are ways by which this can be achieved without costly waste of resources. Dogs runs are expensive and sit idle for long periods in most facilities. For example, it might be better to use tread mills or bull-ring type exercisers which would assure that the animal received its exercise. These are less expensive and occupy less space than runs.

We do not know how much and what kind of exercise is most effective for keeping an animal in optimum health. What do we use for criteria? A certain increase in cardiac output? Elevating the minute volume of respired air by some increment? Different animals have different requirements. There are many research groups in the United States which are qualified and have the capability to conduct studies which would tell us the best kind and how much exercise is needed to keep dogs in optimum condition. That knowledge based on physiological studies should be the basis of any new standard in the law relative to dog runs. This is important information and we do not now have it. The same kind of reasoning should apply to any other standard which might be set to control handling of animals, whether used in research or elsewhere.

DR. HARMISON: At this time I would like to open the floor to the audience for questions and discussions. I hope these presentations have stimulated thought and created the basis upon which directed efforts can be well focused to bring this consideration into direct discussion by all parties concerned. The floor is now open for discussion.

DR. ALDEN E. STILSON, Ohio State University Hospital: I would appreciate it if Dr. Schwindaman could discuss Section 2.28, Part D (of Rules and Regulations) concerning the interpretation of the proper use of anesthetics, analgesics and tranquilizing drugs.

DR. SCHWINDAMAN: To get to Dr. Stilson's question on Section 2.28, Part D regarding the annual report of research facilities certification by the attending veterinarian of the research facility or by the institutional committee of three people, one of whom shall be a Doctor of Veterinary Medicine. I feel that perhaps what Dr. Stilson is inferring in his question is that the attending veterinarian may not have jurisdiction over all experiments being conducted in that institution, yet we're saying that the attending veterinarian or the institutional committee must certify on his annual report that these experiments were conducted with the proper use of the three classes of drugs or in those cases where drugs were not used, that there was a reason for this. It's going to be a problem of the institution itself to set up an internal procedure by which the responsible official for the research facility is going to have to give the veterinarian and/or the institutional committee the authority to see that these experiments are conducted under the provisions of the Act. If they do not give the attending veterinarian or institutional committee this authority, then, and this is only as I see it right at this point, that institution may very well be in violation of the provisions of the Act.

I think that the problem is going to be educating the institutions and the responsible officials to the fact that the attending veterinarian or institutional committee is going to have this authority in order to make such certification. Or the committee or official responsible for that institution is going to have to make that certification himself.

I don't know if this has answered your question entirely, but it is an internal problem that will have to be worked out in many institutions.

DR. STILSON: Well, being a little bit tacky about this, if you will, in a university there are all kinds of colleges. In some areas there are personnel who are quite capable but may not be veterinarians and some cases where there are personnel whose capabilities may be more doubtful, although up to this time they have been using these types of drugs, properly or improperly, we don't know.

The veterinarian is at this point in time being placed in a position of having to certify to what they're doing. It appears that he has got to start making value judgments on these people's capabilities. This puts him in rather a difficult position with respect to the university's total organization and hierarchy, etc., so I believe that we would appreciate something more specific than what you outlined.

DR. CONNER: He would like to have more specific guidelines as to exactly how far the

attending veterinarian's authority should go in regard to the review of the protocol, the procedures, the experiments themselves.

DR. STILSON: Are we to look over their protocol before they do it and approve it or disapprove it?

I, like everybody else in this room, use the Heart & Lung group's word when they certify that something did happen. They want to know it did happen. Now how can I be every place and know that it did happen? The only thing I can do is rely on people in whom I have confidence as to their professional capabilities and their integrity.

DR. SCHWINDAMAN: I think that we certainly appreciate that as highlighting the question of what specific information is needed. I think we all find ourselves in somewhat of a difficult situation in each aspect, the difficulty as reflected by the panel here trying to put into perspective regulations and policy on the one hand as well as trying to deal with some of the very detailed things involved in organizational structure and policies of administration.

I think that's an awfully large question when we talk about how it affects the individual structure and highly personalized, so I think they'd appreciate dealing directly with you. I would suggest that you talk about the details of the structure within your institution possibly after the discussion at the present time.

DR. ESTELLE H. GELLER, Albert Einstein College of Medicine, New York: When a grant is approved by NIH, are we to assume that their criteria have been met by the specific investigator's protocol?

DR. SCHWINDAMAN: If we approve and fund a grant to your institution, can your institution then assume that it has done everything that it's supposed to do? Is that the question?

DR. CONNER: I don't really think you can make that assumption. The responsibility is reallly directed back to the institution. At the moment, this NIH policy is a general broad-based thing that goes on and on. What we ask you to do is tell us how you're going to manage this, there is one or the other of two different routes, either AAALAC accreditation or the existence of a faculty committee. The responsibility is there with the faculty committee. You can assume that that grant has been reviewed by a study section and a PG. Q–56 council, both of which have looked at the same things. They are charged with the responsibility just as well as your committee.

So, in a sense, my answer would be in the affirmative; yes, you can assume that our review has not turned up anything questionable.

DR. GELLER: I have one more question. If in the opinion of the research committee of the institution or the attending veterinarian, something isn't quite right, that unnecessary pain can be avoided, or whatever, can the investigator . . . say, "Well, I didn't know anything about that."

DR. SCHWINDAMAN: The question was is there any punitive action, mainly legal sanction against the experiment, to the researcher who does not follow the suggestions of, perhaps, the institutional committee.

The law itself does not have any particular legal sanction against the researcher himself. The legal sanction would be against the registered research facility, so how this would come to our attention would be another matter. It may be through the attending veterinarian. It may be through the institutional committee. Or it may be through our inspector, making inspections of the premises.

But most assuredly the responsible officials of the institution would know about it first. We're not in the business to have alleged violations. We're not in the business of providing humane care and handling for the animals as provided by the law.

DR. GELLER: I don't mean to imply that any thing's wrong in my institution.

DEAN FRANKLIN, Scripps Clinic & Research Foundation: As an outsider and something of a layman here, I feel that . . . you can't lay down solid guidelines in hospitals and make laws to uphold these guidelines.

DR. CONNER: I think that there are two points we can address, possibly the scientific side of the guidelines. My answer concerns how you organize a facility to implement the guidelines in your institution.

Dale, would you like to further amplify on the scientific aspect of the guidelines?

DR. SCHWINDAMAN: Well, our initial reac-

tion, as far as the administering agency was concerned, was that we did not want to put in our regulations anything so inflexible that it would tie down the investigators, the institutional committees or the attending veterinarians.

I think Congress intended that if the scientific community did not respond positively that most assuredly there could very well be more stringent legislation. I like the approach that they have taken, and I hope that the scientific community will respond in a positive manner. If a particular investigator does not concur with the guidelines as established by the in-house institutional committee, the institution itself will take care of this, without having to take any legal sanctions under the provisions of the law.

Even though I realize that I'm not responding directly to your question, I feel that, at this point, we can utilize the institutional committee and the attending veterinarian to provide the intent of the law without getting into any more stringent guidelines.

KEITH KRANER, University of Missouri: I have a question regarding the definition of some of the animals. I've alternated between feeling that I understand and that I don't understand a number of times.

I guess the question is when is a pig not a pig? For instance, you mentioned that farm animals are not animals currently under this legislation. A pig is a farm animal, but is it always a farm animal? Or is it not a farm animal, for instance, when it's used in medical research? Is it not a farm animal when it's used in zoological research? Or is it, in all cases, not included at this time?

In some situations it can be a rather significant factor when it comes up for documentation in the annual report. It may mean several thousands of animals don't have to be included in one definition, and yet in another may have to be.

DR. SCHWINDAMAN: Well, Dr. Kraner, I can't answer very specifically in saying that at this time a pig is a farm animal and will not be included under the definition of "animal." Some of the confusion comes from the fact that in our definition of "animal," and the legal counsel advised us of this, we had to taken certain wording out of the Act itself and include it in our definition. It became very confusing when we started talking about horses and farm animals that may be used for purposes of improving the breed, or nutrition. The legal people have no hesitancy about saying that farm animals under our present definition are not covered. When we do cover farm animals, we will have to take into consideration all these other qualifying causes that are in the definition, but at this time farm animals are not covered.

DR. AARON LEASH, Case Western Reserve University, Cleveland: Question regarded writing an annual report and types of animals included.

DR. CONNER: The way our policy is currently established, it is restricted to the six species that the USDA law covers. However, if the law takes the anticipated course, and an investigator proposes to an NIH entity a project involving the use of pigs and gets that support, then those pigs are considered experimental animals. They are definitely homothermal, and they would be covered by the intent of our policy.

QUESTIONER (unidentified): We have a considerable number of things to report annually, the number of animals, the facilities where they're used, the names of species, painful experiments, and the use of painful drugs. Will the USDA provide us with forms or are we supposed to write this out in an annual report? Also, since we don't have any forms, are we supposed to be collecting this information now? When does this reporting period begin?

DR. SCHWINDAMAN: Yes, the Department of Agriculture will have forms for this purpose. People in the Office of Management and Budget . . . told us that we did have to have a form for this purpose, so we have devised one. It's very simple, and we hope to have it out to you within the next three months.

Believe me, this is kind of a joke with us too because we had anticipated starting to enforce this law as of December 24th and you can't enforce it without the forms that we don't have yet.

In answer to your specific question, there will be a form for this purpose. The reporting period will be for the calendar year prior to February

1 of 1973 when the first report is due, and it will be the calendar year with the report due on February 1 following the calendar year each subsequent year. In other words, the calendar year 1972 will be the first period covered by your annual report, which is due on February 1, 1973.

NATHANIEL P. CHING, St. Vincent Hospital and Medical Center, N.Y.C.: I have a question concerning manpower. The Agriculture inspector assured me that we can use our local veterinarian without any problem at all.

DR. PARKER: The way I understand the regulations, a veterinarian who is working in the community could be hired. He doesn't necessarily have to be involved with the research, but I think he's sticking his neck out when he signs as a responsible veterinarian of the committee. He's going to have the same kind of responsibility to see that the guidelines have been adhered to. Therefore he should be informed on the research.

There have been programs to train veterinarians in animal care but there are many veterinarians now supervising facilities who have not had formal training. As a professional, it's his responsibility to see that he knows the guidelines and that he and that committee see they're enforced.

DR. CONNER: Dale, do you want to augment the statement?

DR. SCHWINDAMAN: Essentially what Dr. Parker said is true, the institution may have a veterinarian on a consulting basis, a part-time basis or a full-time basis. This can be someone that is in general practice or in small animal practice in the area. I think it behooves the veterinarian who does sign a contract with an institution to take some of these refresher courses and become more familiar with the animals that are used for research and some of the problems that are involved in the use of these animals for research. But yes, a practitioner can be utilized for this purpose.

ROBERT M. LEWIS, Tufts-New England Medical Center, Boston: I'd like clarification on one point. If hogs, or farm animals, are excluded and yet they're used in research projects funded by NIH, they are then included under the NIH policy. Does this not also include rats and mice which are excluded specifically by the Act, but are funded by NIH in a number of different diverse projects?

DR. CONNER: These are difficult points to deal with now because we are in a transitional period. At the moment the NIH policy, as it's written, applies to just the same six species to which the Act applies. But if the law takes the direction that I am quite sure it will, if a man proposes and gets support for a project that will use any kind of warm-blooded species, then you will have to have your university committee look at it or be sure that AAALAC accreditation applies.

I could raise a question here with Dr. Schwindaman; does not the Act make a distinction between animals that are and are not being used for production research? Isn't there a distinction in the law in that respect that would apply to pigs as well as horses, calves, etc.? I thought that this was the case.

DR. SCHWINDAMAN: Well, the law itself did make a distinction between animals who were going to be used for production research and animals that were going to be used strictly for basic research protocols and designs. However, when we wrote this definition in our present regulations, we wanted to exclude all farm animals across the board, so this was the way the attorneys told us to write it; put the commas in the right place, conjunctions in the right place, and the periods in the right place, so that legally it would be interpreted to mean that farm animals are excluded across the board.

Now, as I mentioned previously, when warm animals are designated by the Secretary, then we will have this problem. I can see that it's going to be a problem of determining when farm animals are used entirely for basic research or when they're used for purposes of improving their health or some other reason.

DR. HARMISON: I was wondering, since we're defining what a pig is and when he's a pig, could you tell me the difference between production research and research?

DR. SCHWINDAMAN: Well, I'd like to give you the advantage of our present thinking in conjunction with a USDA committee that we convened for this purpose prior to writing the regulations. We felt, and we still feel, that, at

such time when farm animals are included, if a university and the Agriculture experimentation station has a herd of farm animals in a pasture where they have plenty of room, and are not confined in a small enclosure, they would not be covered, even though they may be involved in some breeding or nutrition research. But if they are brought under laboratory conditions, then they would be covered.

Now I realize there's a gray area here and there's a lot to be discussed and determined here before we make our final decision, but this is our thinking at the present time.

MR. FRANK J. MCMAHON, Humane Society: Dale, you stated that the annual report is not public information and will not be. I assume by that you mean it will be privileged information. I would like to know whether that decision has already been made by the USDA attorneys or that this is something you're just speculating.

DR. SCHWINDAMAN: The decision has been made by the the USDA legal counsel that the intent of the law, as worded, is that this would be privileged information and not available for release to the public until such time as it is released by the President of the Senate or the Speaker of the House.

LEONARD LEWIS, CIBA Pharmaceutical Co., Morris, N.J.: I was wondering if you would reiterate your statement on rats and mice being excluded. Did I understand that right? I was asked to find out about the housing of animals. There would seem to be some question about putting certain rats and mice in the same room, or not putting mice and hamsters in the same room. Could you comment on this?

DR. SCHWINDAMAN: The question was, first of all, are rats and mice covered under the definition at the present time. No, rats or mice are not covered, they are excluded across the board.

The second part of the question was the separation of species. In developing our law, one of the requirements was that we develop standards for separation of species. This means that you can have species in the same room, but you cannot have them in the same primary enclosure. So, you could have several cages of rats, several cages of mice, and several cages of guinea pigs and hamsters in the same room under the same environmental conditions but not in the same primary enclosure.

Either MEARL A. KILMORE or C. J. STARCH, College of Osteopathic Medicine and Surgery, Des Moines: I'd like to address this question to Dr. Conner or somebody from NIH. When you say that grantees must follow the recommendations of the *Guide for Laboratory Animal Care and Facilities*, do you mean, for example, that all cat cages must be 24" high as is stated in Table I on page 27 in that *Guide*, or do you mean that, in effect, we must have cages that correspond exactly with this *Guide*?

DR. CONNER: I'm not a DVM and not a real authority on what's in that book, but from my own reading of it, these are recommendations. I don't think that we would expect your committees to tie the investigators down to the millimeter.

THOMAS F. IMLAY, Animals for Research, Murray, Utah: The question I would like to ask regards shipping, as a dealer, of pre-conditioned calves intrastate and interstate. Are there guidelines for regulations in effect that we should use as we have on our dogs and some of our other animals?

DR. SCHWINDAMAN: At the present time, Bovines will not be covered at all.

QUESTIONER (unidentified): I think our biggest problem is going to come in the field of toxocology. How are we going to establish procedures avoiding pain?

DR. NORMAN: As a clinician, I've been concerned about many of the questions that have been asked. I'll come back to something Dr. La Farge will appreciate. There was a book written, I think, in the mid-fifties by Korzibski in New York that referred to a science and sanity and had to do with semantics. I hope Lowell will excuse me for saying this, but NIH doesn't exist because we are NIH. We're in a position to set up a framework and a mechanism for having the "intercourse" involved in this and sometimes you have to relax and enjoy the event. The important thing is that, at this stage, the scientific community has a major responsibility to define, interpret, and become a responsible voice I think that is a real responsibility that we're facing.

DR. CONNER: From the point of view of NIH and HEW, I wanted to reinforce what Dr. Norman said. As I've said so many times, our policy is in a state of flux. It now applies only to NIH, It's about to be broadened to cover the whole Department. Please give us your comments. Most of you have seen NIH policy.

DR. J. MOOR-JANKOWSKI, New York University School of Medicine: Since you have asked for comments, I would like to make a comment which was in my mind during this discussion. Dr. Gorsky and I represent an institutional laboratory which, for the past five years, has established a policy as you have discussed here. We have a utilization committee, and we have had close to 200 scientists working in our laboratory. We've never had the utilization committee say no because of inhumane treatment of an animal, and we were able to help scientists achieve humane care of the animals. However, we have a major problem that puts the shoe on the other foot. I can enumerate a number of grants in which research was approved but the animal care funding was cut. I heard Dr. McPherson say that he got $1.5 million to upgrade animal care facilities in this country. If it wouldn't be so sad, I would say that this is laughable. Last year I toured various industrial and university facilities in Europe. A one-year-old dog bred for the German, or for the Swiss industry cost in purchase value of the money, between $500 and $800. To be more specific, I think we were very impressed by the conference which the Heart Institute got together, and we are quite a representative group. I think that maybe the gentlemen could carry the message and I am sure that most people here would approve. There are two problems. One is that the funding for the animals is not adequate. The second problem involves blood grouping research. I had a site visit by immunologists who told me that I can do plasmapheresis on four human patients with one nurse in a morning, but I must have three technicians to do plasmapheresis on a chimpanzee. If site visitors come and decide about programs which involve the use of animals, there should be somebody in the group with sufficient authority who knows how the work with the animal is carried out.

In conclusion, I think that if there is any way of bringing to the higher echelons of NIH these two recommendations, and if people here agree, may they just say so. One is that there should be more money appropriated for we will be breaking the law if we do not have the funds to upgrade our facilities. Secondly, site visitors who come to discuss and evaluate programs working with animals should have people who are knowledgeable in animal experimentation, even if it is not in the research program.

DR. CONNER: I think he's made a very good comment. In these projects that you say that direct funds were cut for animal maintenance, did you inquire from NIH or whatever HEW entity it was that made this judgment as to why? You're entitled to know why the budgets were altered or in the case where the application was flatly disapproved, you're entitled to know the reason why.

DR. MOOR-JANKOWSKI: In all cases I've been asked if this could be done more cheaply. Additional technicians are very expensive in New York.

DR. CONNER: Those technicians' salaries were the primary bone of contention then, is that it?

DR. MOOR-JANKOWSKI: If you want to maintain twenty-four hour a day care for each animal, it costs you much more than if you have just one technician with the animal.

DR. CASS: I want to thank you, Dr. Moor-Jankowski, for raising the same question that I tried to allude to when I spoke. The animal maintenance and support and the setting in which you're doing your work is considered main research, so when a research grant comes to our institutions or to the VA, the investigator is told that an animal is a supply item and a per diem cost is all that's necessary to cover it. Yet the new NIH survey during 1968 felt that the per diem charges covered in fact only between 40 and 60% of the actual cost of the operation; this is all published.

ANN FREE, Washington Star: I write about animals often, and I'm a little confused, Dr. Schwindaman, with the copy of the Rules and Regulations about farm animals. Could you clarify this? In listening to the papers so many sheep, bovines and horses are being used. Surely they couldn't be exempt from these regulations. What about a horse for serum?

DR. SCHWINDAMAN: I can understand, Mrs. Free, why you're confused because many of the rest of us are, too. Let me just take a moment to read the definition of animal as we have it in the Regulations . . . "Animal means any live or dead dog, cat, monkey (see PL 91–579 § 84, Statute 1561, non-human primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal being used or is intended for use for research, testing, experimentation, or exhibition purposes or as a pet." There, you have the entire content of the term "animal." Then we say that the term excludes birds, aquatic animals, rats and mice, and horses and other farm animals and this is where the confusion comes in. We say, "such as, but not limited to livestock or poultry used or intended for use as food or fiber, or livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber." It's the clauses after "such as" that confuses everyone. I must admit that it confuses me, but I do have to accede to the attorneys in that the first sentence covers the animals, and the purpose for which they will be used, while the second sentence excludes animals. They feel it excludes farm animals across the board.

ANN FREE: It would seem to me that the attorneys should take a second look at this because it says specifically here that these animals, as long as they're used for "food and fiber" and actual agricultural farm purposes, are not covered. You know as I do that this was put in the law because this came through some Agriculture committees and they didn't want to have their toes stepped on. That's the clear intent. Dr. La Farge and many others are using calves and sheep and so on, and they readily admit that these farm animals, when they are in the laboratory, should fall under the purview of this law. So I think it would be a very good idea to regroup this.

DR. SCHWINDAMAN: When farm animals are included as an animal by the Secretary, then they will be included under the law. The farm animals that are actually under research will be included at that time. Again, we had to use these clauses because it was in the Act itself and it did nothing but really confuse the issue, because we placed them in the Regulations. If we could have just stopped with the exclusions and said "horses and other farm animals," I don't think there would have been any confusion at the present time.

ANN FREE: Does this mean then when they're in research, that they're under the law?

DR. SCHWINDAMAN: No, it does not mean that when they're in research they're under the law at this time. When farm animals are included as an animal, then they will be.

ANN FREE: Well, Dr. Schwindaman, it doesn't make a bit of sense.

DR. SCHWINDAMAN: I can appreciate your feeling on it.

DR. LA FARGE, Boston: I have to rise to that. I can't help wonder what Tennessee Williams is going to do now with his cats on hot tin roofs. I think that one of the problems that you've raised is a very realistic one. I think it's a practical solution that's been offered, i.e., to limit the number of animals until the whole concept of the law is started and is carried through long enough so that we know what the effect is going to be. I don't think the intention of the law is to suppress research or to try to make them jumpy about whether a inspector is going to walk in the middle of a somewhat dubious experiment. I think it's rather to try to encourage investigators to do what Jack and other members of the panel have said and that is to try to conduct their research in such a way as to fulfill the best principles of investigation whether it be human or non-human.

Good research is conducted along lines that are spelled out in medicine by good research principles, irrespective of the species. The best animal experiments done in this country are done from the start to the finish under the principles that guide good thoracic surgery, good medical care, or good pharmacology. Now it doesn't really take a lot of legislation. I think that you're going to have to leave it up to the good conscience, at least for the time being, of investigators. Apparently you have a very low opinion of us to think that we don't deal with animals like calves in a manner which befits not only their dignity but our own. I have a very strong feeling, and I think most investiga-

tors do, that these animals are not only our livelihood but our intellectual stimulation in life as well. I just can't help feeling that this program envisioned by the USDA and by the NIH not only regulates, in the sense that they are trying to make up for the deficiencies of those people who have abused the privilege of dealing with animals in the past, but also encourages people throughout the length and breadth of the research world to deal with animals according to the best possible principles of medicine. I just cannot help but feel that investigators across the land, particularly in the university centers and in animal laboratories who have good conscience, conduct their research properly and who deal with their animals with dignity are by far in the majority.

DR. HARMISON: I think we would certainly underscore the point that research is totally dependent on exquisite care, and this is not something added on to research efforts. It's not an adjunct, it is an integral part of the research. As Dr. Conners pointed out, it is the policy, intent and desire of the program to review these matters as they relate to the NIH supported efforts being looked at in a broader context, by the entire Department of HEW.

QUESTIONER: (Inaudible)

DR. SCHWINDAMAN: Yes, the Regulations and Standards do apply to all these animals even though it's for teaching purposes. The Act itself specified for teaching purposes.

QUESTIONER: (Inaudible)

DR. SCHWINDAMAN: No, the Regulations and the Standards do not require a quarantine period for the animals. This is entirely up to the institution itself as to whether they have a quarantine period for the animals coming into their institution. Now perhaps the holding period you're thinking about is the one placed upon the dealer in animals where he has to hold dogs or cats five days from the time he acquires them. This only applies to dogs and cats, by the way.

ANTHONY V. SEABER, Duke University: I'm an Englishman, and I've been through all this before in 1950 and 1960. I'd like to remark to the press and the receptionist that you must remember we have different lingo. This lingo is very important when you report. I'm sure you heard a very elegant paper given where fellow said he had some heaps of cats in the corner. Now, don't go out and think that we just have heaps of cats in the corner. This is something you must remember when we talk, and you come to our meetings; you must remember we have lingo that you probably don't understand. I did want to bring this point out. In England we have this problem. The ASPCA seems to have a way of coming up here with momentum. In 1952 I was a fox hunter and you almost stopped my fox hunting because somebody got very stupid about it and misinterpreted what we were talking about. I think this is a point for NIH to make. They should give us guidelines and nomenclature so that the public knows what we're talking about and so that the press does not manipulate our experiments.

DR. HARMISON: I certainly appreciate your point. I think communications is one of the major and critical problems.

JEROME B. SENTURIA, Cleveland State University: You said something about any animal that had at one time been wild or something. How do we interpret those guidelines with regard to animal models which are really wild animals?

DR. SCHWINAMAN: The question concerned animals that are normally found in the wild and caught in captivity and are presently in captivity or raised in captivity. They're going to be covered across the board, even though they're used for research purposes. Animals that are trapped in the wild, perhaps including rats trapped in the wild, although we've not had a legal determination on this because of the term rats and mice, but definitely other members of the rat family that are trapped in the wild and used for research purposes will be covered. Your woodchucks will be covered. Armadillos will be covered. Any animal that is normally found in the wild will be covered. Once an animal is designated by the Secretary, it's covered across the board. That's the reason I've used that term several times, "across the board."

DR. SENTURIA: How do we know that the size of the cage meets the guidelines, if there are no guidelines?

DR. SCHWINDAMAN: This is a very good point and I'm surprised it has not been brought up before. You'll notice in our statement of con-

siderations that there were two things to which we had to address ourselves. One is the exercise requirement that Dr. Parker mentioned. The other is the space requirements for the animal species: dogs, cats, guinea pigs, rabbits, hamsters, and non-human primates. It is true that at the present time we do not have specific space requirements for all these and other large numbers of animals. The reason that we don't have them is because there is not enough information available at this time for more specific space requirements. There is information coming in, we hope to get it assimilated; and if something specific comes out, we can place this in our standards. I might say, just as an observation, that when you publish regulations and standards, you sit down and you write them as practically and with the interpretation that you make of the law. When the legal people get a hold of them, they sometimes rearrange them, put commas in different places and so on. However, one of the things they mentioned was that they felt that according to the Act, we were going to have to have more specific requirements for all animals, other than the six species. What we're proposing for those two areas, exercise and space requirements for all these other animals, is that within sixty days from December 24, 1971, we will publish in the February register our intent to solicit data, views, arguments, anything that the public has concerning these two issues. We will probably give a period of sixty days. After that time, we will assimilate this material and then have meetings of representative groups to review it, evaluate it, and decide just what is a practical standard here. We do not have enough information at hand at this time to develop practical standards? Until such time as we do, we're going to have to live with these, very broad guidelines for standards as far as all the other animals are concerned.

DR. HARMISON: I certainly want to express on behalf of the panel our appreciation for seeing something in excess of five hundred people attend this non-technical adjunct to the more scientific portion of the program. I think it is an inherent responsibility and a challenge to the scientific community to accept and deal with it and to help bring forth the standards to mold and shape an environment in which we can make progress. In closing, I want to express my deep appreciation to the panel for their efforts and participation in this program.

U.S. GOVERNMENT PRINTING OFFICE: 1974 O—469-414

NIH LIBRARY
3 1496 01062 4396

DHEW Publication No. (NIH) 72-335

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health